JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45 WASHINGTON, D. C., NOVEMBER 1, 1932 No. 9

A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA CORONATA[1]

By RUTH F. ALLEN [2]

Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and *Associate in Agronomy, California Agricultural Experiment Station*

INTRODUCTION

Since the announcement by Craigie in 1927 of heterothallism in the rusts (*11*) [3] and his subsequent investigations confirming and extending his observations (*12, 13*), work has gone steadily forward on this subject.

Waterhouse (*26*), Stakman, Levine, and Cotter (*25*), and Newton, Johnson, and Brown (*22*) hybridized physiologic forms of *Puccinia graminis* Pers. and found in the progeny physiologic forms of the rust new to science.

Hanna (*16*) published a preliminary account of cytological studies of *Puccinia graminis* showing that the isolated monosporidial infection remains haploid and produces haploid aecia. When spermatia (pycniospores) of opposite sex are transferred to it, the spermatia germinate, and soon after this the sporophyte generation originates in the aecium in the manner described by Christman (*8, 9*).

The writer (*1*), in a paper covering the same ground, found that in the infection of *Puccinia graminis* to which spermatia have been transferred the sporophyte generation begins earlier. Mycelial cells containing from one to five nuclei are found at the spermogonia, at various points in the mycelium, and in the young aecium before the sporogenous layer is differentiated.

Andrus (*3*), in a cytological study of *Uromyces appendiculatus* (Pers.) Fries on beans and *Uromyces vignae* Barclay on cowpeas, found in the aecial primordia certain uninucleate 2-legged cells, which are considered equivalent to eggs. Each is joined to a "foot cell," and from each a hypha, trichogynous in nature, grows upward, branching as it grows, to the upper epidermis (and to a lesser degree to the lower), where the tips project into stomata or between epidermal cells. Spermatia on the surface of the leaf enter the ruptured tips of

[1] Received for publication Mar. 2, 1932; issued November, 1932. Cooperative investigations, Agricultural Experiment Station of the University of California and Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Acknowledgments are made to W. P. Fraser, of the University of Saskatchewan, Saskatoon, Canada; M. Newton, of the Dominion Rust Research Laboratory, Winnipeg, Canada; L. D. Leach, of the California Agricultural Experiment Station; and H. B. Humphrey and H. C. Murphy, of the Division of Cereal Crops and Diseases, for spores of *Puccinia coronata;* to H. C. Murphy and L. D. Leach for seed of *Rhamnus cathartica;* and to the members of the divisions of agronomy and genetics of the University of California for courtesies extended during the work.

[3] Reference is made by number (italic) to Literature Cited, p. 540.

these trichogynous hyphae, and their nuclei travel through them to the egg, which then, either directly or after further proliferation, gives rise to spores.

In a similar study of *Puccinia triticina* Eriks. the writer (*2*) found that the isolated infection of monosporidial origin remains haploid. Spermogonia form in about equal numbers at both surfaces of the leaf. While spermogonia are developing, hyphae grow into stomatal apertures at the lower surface of the leaf or between epidermal cells at either leaf surface. Aecial primordia form near the stomatal hyphae. When spermatia from another and different infection are applied to the surface of an infected area, spermatial nuclei may enter at any exposed hypha tip, initiating there the sporophyte generation, which then spreads quickly from the point or points of origin and soon permeates the aecia. The spread may be by growth, perhaps also by "diploidization," as described by Buller (*7*). Multinucleate cells are common during this development, both in the mycelium and in the aecia.

The theories of Blackman (*5*, *6*) and Christman (*8*, *9*) as to the origin in ontogeny of the sporophyte generation in rusts are somewhat at variance with each other. Recent advances in our knowledge of heterothallism and the function of spermatia in this group have brought both of the older theories under question. It was in the hope of obtaining further light on the subject that a cytological study of *Puccinia coronata* Cda. was undertaken.

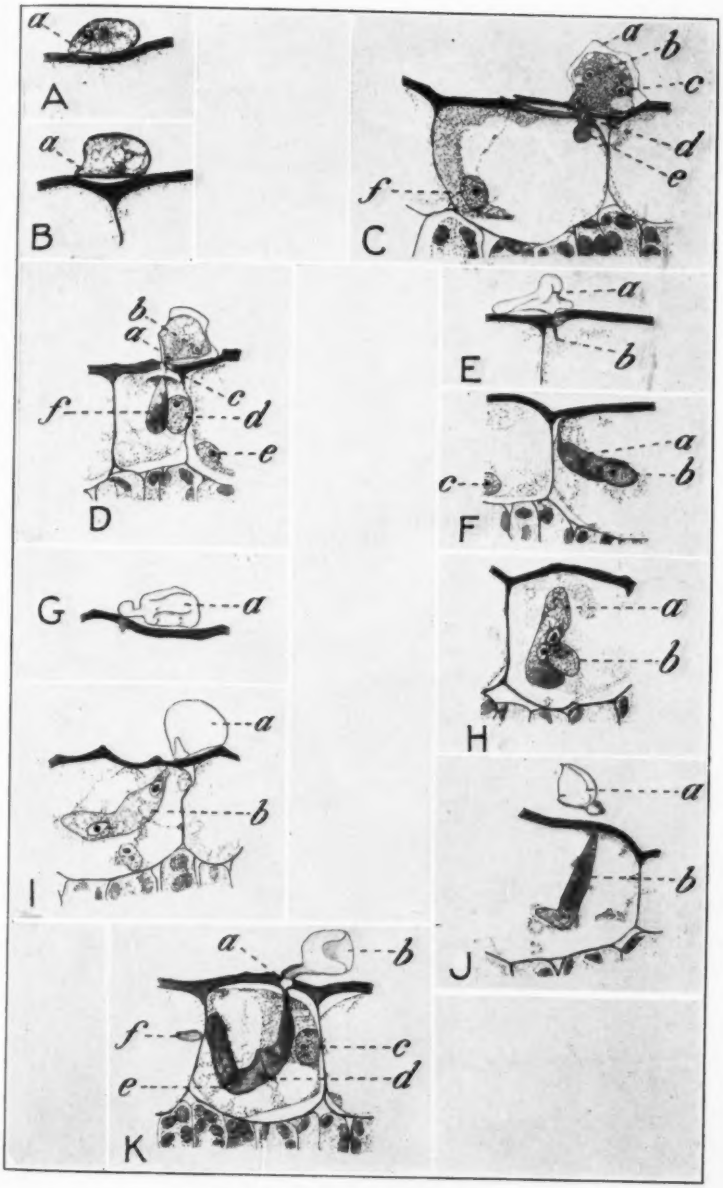
MATERIALS AND METHODS

Spores of *Puccinia coronata* were stored, until time for use, in loose-mesh cloth bags on the ground in a partly shaded spot, or in the head house (potting house). Buckthorn plants (*Rhamnus cathartica* L.) were grown in the greenhouse and used when they were from 4 to 10 inches high.

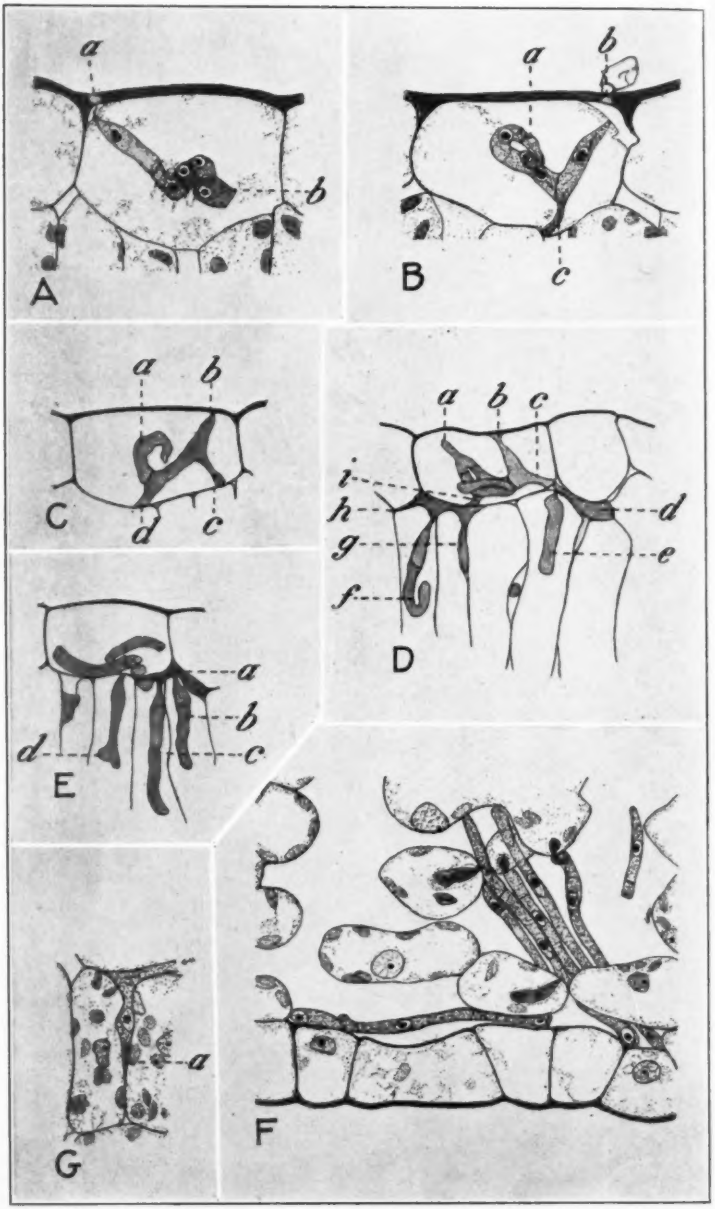
In inoculating, watch crystals were filled with mud. Bits of rusted oat leaves were soaked in rain water several hours and sprayed with an atomizer, then pressed into the mud with the spores exposed, and sprayed again. A bit of wet sphagnum was wrapped around the base of each buckthorn seedling. Tall glass tumblers with the bottoms removed and the sides lined with wet paper were placed over the seedlings, and the mud-filled crystals were used as lids. A layer of wet paper was folded down over the top of each tumbler and held in place with a rubber band. The whole was placed under a greenhouse bench for 48 hours, then uncovered and placed on the bench. The plants were kept covered with tarlatan cages to exclude insects.

EXPLANATORY LEGEND FOR PLATE 1

A.—Uninucleate sporidium of *Puccinia coronata* on the surface of a Rhamnus leaf, pushing out a beak at *a*.
B.—Uninucleate sporidium, with narrower beak (*a*).
C.—Beginning of infection: *a*, Sporidial wall; *b* and *c*, nuclei in cytoplasm; *d*, pierced epidermal wall; *e*, entering cytoplasm; *f*, host nucleus.
D.—Stage of entrance: *a* and *b*, Nuclei in the sporidium; *c*, pore in the wall at the point of entrance; *f*, club-shaped mass of fungus plasm in cell; *d* and *e*, host nuclei.
E.—One-day infection: *a*, Collapsed sporidial wall; *b*, entrance tube.
F.—One-day infection: *a*, Binucleate fungus cell in epidermal cell; *b* and *c*, host nuclei.
G.—One-day infection: *a*, Collapsed sporidial wall.
H.—One-day infection: *a*, Binucleate fungus cell in epidermal cell; *b*, host nucleus.
I.—Two-day infection: *a*, Empty sporidial wall; *b*, binucleate fungus cell.
J.—Two-day infection: *a*, Sporidial wall; *b*, trinucleate fungus cell inside.
K.—One-day infection: *a*, Entrance pore; *b*, sporidial wall; *d* and *e*, septa of 3-cell primary hypha in epidermal cell; *f*, branch from primary Hypha; *c*, host nucleus. (All × 1,130.)



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

Material was fixed daily for the first three weeks and at longer intervals from then on until the death of the fungus. Several fixing solutions were used. Flemming's weak was the most trustworthy, but Flemming's medium was equally good with older material. After remaining 36 to 48 hours in the fixing fluid, the material was washed, dehydrated, and embedded in 50° paraffin. The principal stains used were safranine and methylene blue. These are excellent when the slides are fresh. Prolonged exposure to light, however, fades the blue stain.

EXPERIMENTAL DATA

ENTRY OF THE RUST

Twenty-four hours after the Rhamnus plant was placed in a damp chamber, with the rusted oat leaves above it, some of the teliospores germinated and produced and discharged the sporidia, or basidiospores. The sporidia fell on the leaves, where some of them germinated and entered the host, forming hyphae of several cells within the epidermal cells. The steps in this last process have been studied.

The sporidium is a small, thin-walled, short-lived spore containing a single nucleus and cytoplasm with scant food supply. In the presence of moisture it germinates promptly, forming a short, broad process at one end (pl. 1, A, *a*)[4] or a narrower beak (pl. 1, B, *a*), or, more rarely, a short germ tube almost equaling in length the diameter of the spore. The tip of this beak becomes closely applied to the epidermal wall, and entry is effected at that point. By the time entry has begun, the sporidial nucleus has usually divided into two. (Pl. 1, C, *b*, *c*; D, *a*, *b*.)

In the uredinial generation the spore produces a long germ tube, often many times the diameter of the spore in length, which grows to the nearest stoma and enters through the stomatal aperture. That method of entry is not feasible here. No stomata have been observed on the upper surface of Rhamnus leaves, where at least 99 per cent of the entries take place. The fungus enters directly through the thick outer epidermal wall. The means by which this wall is perforated, whether chemical or mechanical or a combination of the two, has not been determined. The opening in the wall is large enough to be visible under the microscope. (Pl. 1, C, *d;* D, *c;* E, *b;* K, *a;* pl. 2, A, *a;* B, *b*.)

The entering cytoplasm forms a rounded mass within the epidermal cell (pl. 1, C, *e*), which soon expands into a club-shaped body extending toward the inner face of the cell. (Pl. 1, D, *f*.) This curves so that the distal end lies free in the center of the epidermal cell. (Pl. 1, F, *a*.) In this case the empty collapsed sporidial wall (pl. 1, E, *a*)

[4] For the sake of uniformity and clearness, the drawings are oriented in the plates as the tissues are in the leaf, i. e., with the tissues nearest the upper surface of the leaf uppermost in the drawing.

EXPLANATORY LEGEND FOR PLATE 2

A.—Two-day infection: *a*, Entrance pore; *b*, 5-cell primary hypha. × 1,120.

B.—Two-day infection: *b*, Entrance pore in sporidial wall; *a*, 4-cell primary hypha with one branch (*c*). × 1,130.

C.—Three-day infection (outlines only): *a* and *b*, 3-cell primary hypha with two branches (*c*, *d*). × 1,130.

D.—Three-day infection (outlines only): *b*, Primary hypha with branches (*a*, *c*, *i*) giving rise to subepidermal hyphae (*d*, *h*), a hypha (*g*) growing down between palisade cells, and haustoria (*e*, *f*). × 1,020.

E.—Three-day infection (outlines only): *a*, Subepidermal hyphae; *b*, 2-cell haustorium; *c*, 3-cell haustorium; *d*, 1-cell haustorium. × 1,020.

F.—Four-day infection from an entrance through the lower epidermis of leaf. Hyphae growing freely in air spaces. × 1,020.

G.—Detail of 4-day infection: *a*, Hypha forcing a passage between palisade cells. × 1,020.

and the entrance tube (pl. 1, E, *b*) were found in the next section. Similar examples are shown in Plate 1, G, H, and I.

The transferred protoplasm of the sporidium is now an elongated, often sausage-shaped mass in the interior of the host cell. It is still unicellular and binucleate. As entry progresses, the delicate sporidial wall outside collapses irregularly. The host nucleus, normally lying against the inner wall of the epidermal cell (pl. 1, C, *f;* D, *e;* F, *c*), is now commonly in contact with the fungus (pl. 1, D, *d;* F, *b;* H, *b;* K, *c*).

A septum may now divide the young primary hypha into two cells, or there may be another nuclear division before septation. In Plate 1, J, the cell (*b*) contains three nuclei. Plate 1, K, shows a 3-cell hypha formed from such a trinucleate cell. The fungus is overstained, so that the details of the contents are vague, but the septa (pl. 1, K, *d*, *e*) are unmistakable.

The primary hypha usually develops further by apical growth, becoming 4-cell, 5-cell, or even 6-cell, and irregularly bent or coiled within the host cell. In Plate 2, A, the primary hypha has five cells, and from the truncated appearance of the tip (*b*) at least one other cell was lost in sectioning.

DEVELOPMENT OF MYCELIUM

On the second day, the primary hypha branches, and the subepidermal region may be invaded. With few exceptions each cell of the primary hypha branches in turn, beginning with the oldest. In Plate 2, B, only the first cell has branched (*c*). In Plate 2, C (drawn in outline only), the first and second cells have branched (*c*, *d*). In Plate 2, D (outlines only), branches from the first, second, and third cells have formed at *c*, *i*, and *a*, respectively. Usually these branches of the primary hypha grow directly toward the inner wall of the epidermal cell and push through into the subepidermal area. More rarely, a branch may enter the next epidermal cell (pl. 1, K, *f*) or even emerge upon the outer surface of the leaf.

In entering the subepidermal region, the fungus encounters mechanical obstruction. The palisade layer of the Rhamnus leaf is very compact and is closely applied to the epidermis. Intercellular spaces are few and small. Plate 2, D, already referred to, shows (outlines only) a central section through a 3-day infection. Two branches of the primary hypha (*c*, *i*) have passed through the inner wall of the epidermal cell and formed the intercellular hyphae (*d*, *h*) by separating the host cells and growing between them.

Whether this splitting of host-cell walls is achieved by mechanical or chemical means is not clear, but it is probably the former. There

EXPLANATORY LEGEND FOR PLATE 3

A.—Median section of 4-day infection: *a* to *f*, Subepidermal mycelium; *c*, primary hypha; *b*, *d*, *e*, hyphae growing down, the last reaching intercellular spaces below (*g*, *h*). ×640.

B.—Detail of 4-day infection: *a*, Flat view of wedge-shaped hypha forcing passage between palisade cells. ×1,020.

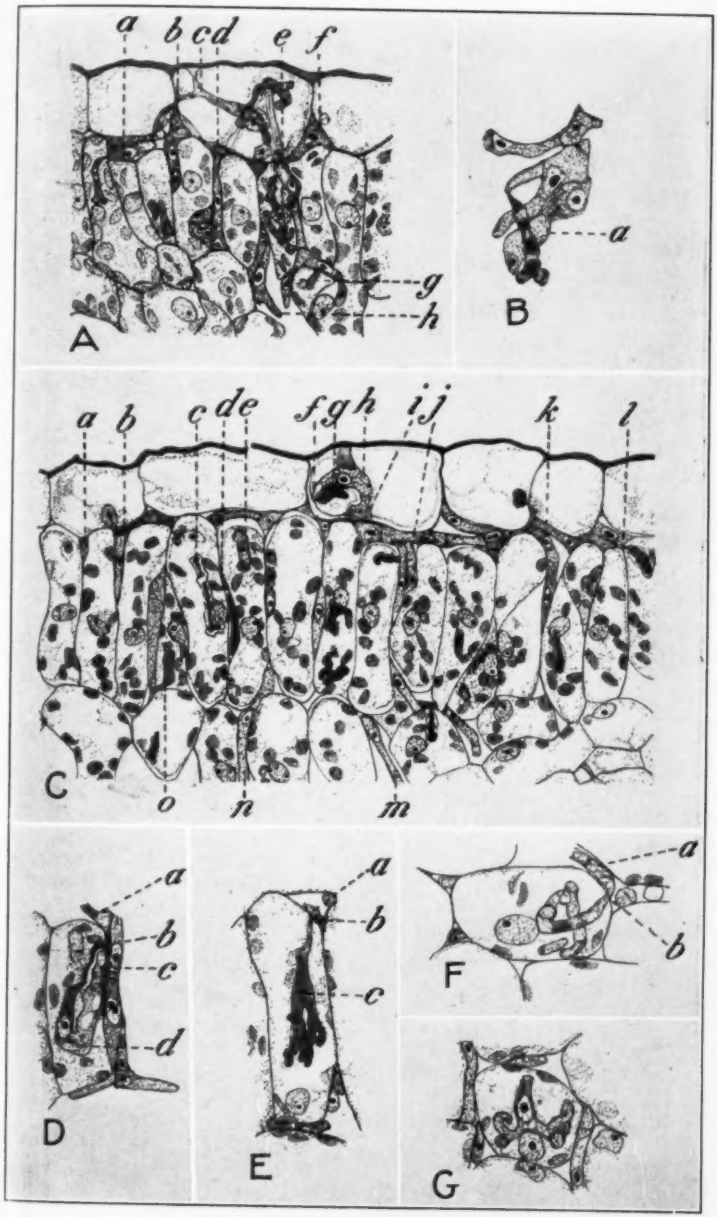
C.—Central section of 6-day infection: *a* to *l*, Subepidermal mycelium; *f*, *g*, *h*, and *i*, remnant of primary hypha; *a*, *b*, *d*, *j*, and *k*, hyphae between palisade cells; *m* and *n*, hyphae in air spaces below palisade; *c*, *e*, and *o*, haustoria. ×730.

D.—Detail from 4-day infection: *a*, Haustorium initial; *b*, entrance to host cell; *c* to *d*, branchless bent haustorium. ×1,020.

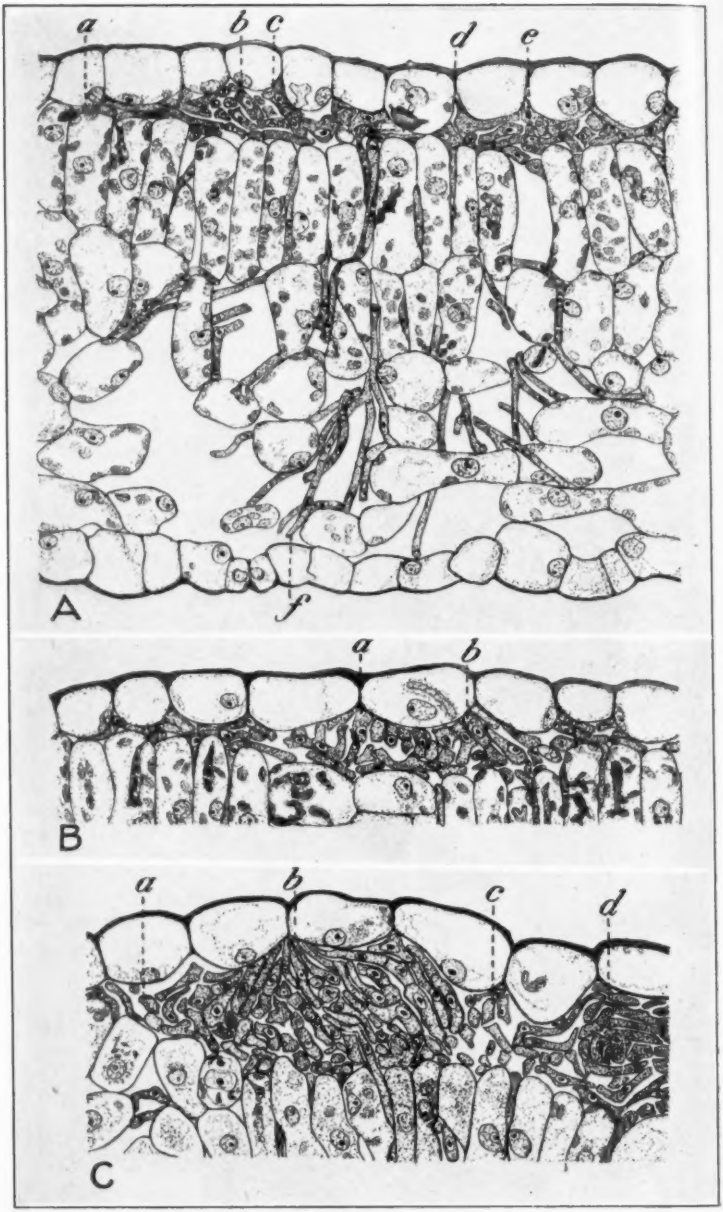
E.—Detail from 7-day infection: *a*, Haustorium initial; *b*, entrance; *c*, dichotomously forked haustorium. ×1,020.

F.—Detail from 4-day infection: Cytoplasm visibly continuous at *b*, between haustorium initial (*a*) and haustorium. ×1,020.

G.—Detail from 4-day infection showing dichotomously forked haustorium in mesophyll cell. ×1,020.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

is no evidence of swelling and softening of the middle lamellae of the walls such as one would expect if they were being dissolved. Moreover, the advancing tip of the hypha often forms a flattened, sharp-edged wedge, well fitted for forcing a passageway. The edge view of such a hypha is seen in Plate 2, D, at *h*; Plate 2, G, at *a*; and Plate 3, C, at *a*, *b*, and *k*. The appearance in flat view is shown in Plate 3, B, at *a*.

The initial intercellular growth of the rust is between the epidermal and the palisade layers. In order to reach the more open spongy mesophyll of the leaf, the fungus must either grow down through the palisade cells or force a passage between them. In Plate 2, D, both methods are being essayed. At *g* is a hypha making its way down between two palisade cells. At *e* and *f* are intracellular growths intermediate in character between haustoria and hyphae. These may be 1, 2, or 3 cells in length. (Pl. 2, D, *e*, *f*; E, *b*, *c*, *d*.) Although they are like hyphae in appearance, they are probably to be regarded as haustoria. No case of successful passage through a palisade cell into the air spaces beneath has been noted.

Rarely, a sporidium enters through the lower epidermis of the leaf. In this case the mycelium reaches spongy mesophyll at once (pl. 2, F) and spreads freely through the air spaces.

A slightly later stage of development is shown in Plate 3, A. From the primary hypha (*c*), now more or less disintegrated, subepidermal hyphae have formed and spread from *a* to *f*. Branches from these are working their way down between palisade cells at *b*, *d*, and *e*, and at two points (*g*, *h*) have reached the freedom of larger air spaces.

The tendency to spread just beneath the epidermis persists as development continues. Plate 3, C, shows a central section through a 6-day infection. This material was fixed early in the season when the fungus grew slowly. Later in the spring a 6-day infection would be forming spermogonia. The primary hypha (pl. 3, C, *h*) is still recognizable. At *f* and *i* are points of entry into subepidermal spaces. There is now a continuous mat of subepidermal hyphae, from *a* to *l*, still only one cell thick for the most part, but in places thicker (*j*, *k*, *l*). There are several hyphae working down between palisade cells (*a*, *b*, *d*, *j*, *k*) and a few deeper in the leaf (*m*, *n*). The cells of the mycelium are regularly uninucleate.

Not all the haustoria formed in palisade cells are hyphalike. Plate 3, D, shows one of quite a different type. The haustorium initial (*a*) effected an entry into the host cell at *b*, and the haustorium has grown down to *d* and doubled back to *c*. It is a single unbranched cell, with the uneven diameter and irregular outline common to haustoria. Some of the haustoria fork dichotomously at the inner end. Cases of repeated dichotomy are figured in Plate 3, E and G. A distinguishing trait of gametophytic haustoria of crown rust is the presence of cytoplasm (but no nucleus) in the haustorium initial after the haustorium is fully formed. (Pl. 3, D, *a*; E, *a*; F, *a*.) Another difference is the comparatively large pore formed in the host cell wall at the point of entry. (Pl. 3, D, *b*; E, *b*; F, *b*.) The cytoplasm of haustorium and haustorium initial may be visibly continuous.

EXPLANATORY LEGEND FOR PLATE 4

A.—Transverse section of leaf through center of 6-day infection: *a* and *e*, Subepidermal stroma; *b* and *e*, beginnings of spermogonia; *c* and *d*, receptive hyphae; *f*, mycelium spreading through air spaces below.
B.—Slightly later stage (6-day infection) with spermogonia started at *a* and *b*.
C.—Six-day infection: Young spermogonia organized at *b* and *d*; looser broken hyphae between, at *a* and *c*.
(All × 640.)

Several cases have been found in which two or even three contiguous epidermal cells contained primary hyphae. The mycelia developing from these became intimately interwoven. While, on the basis of heterothallism, some of these double infections may be both (+) or both (−), it is unlikely that all of them are. In the cases of treble infection, the chances that all three are of the same sex are still further reduced. Yet a careful survey of these multiple infections shows regularly uninucleate cells. There is no apparent attraction between (+) and (−) elements at this stage.

SPERMOGONIA AND RECEPTIVE HYPHAE

The mycelium grows rapidly, permeating the intercellular spaces of the spongy mesophyll (pl. 4, A, *f*) and pushing out farther between the epidermis and the palisade (pl. 4, A, *a*, *e*). The dominant growth is still just beneath the epidermis. The subepidermal mat, or stroma, has spread marginally and thickened at the center. In spots it is now four or five cells thick (pl. 4, A, *b*, *e*), and although there is no recognizable organization of the hyphae here, it is at such points as these that the spermogonia originate. Throughout the area of the mat the epidermis has been separated from the palisade and is being lifted by the growing fungus beneath it.

At this time several centers of development appear in the stroma, in each of which a small circular patch of cells pushes up a dense growth of short, upright hyphae, slanted somewhat toward the center of the patch. Plate 4, B, *a* to *b*, represents such a center. This is the beginning of a spermogonium.

A somewhat later stage in the development of the spermogonium (also from a 6-day infection) is shown in Plate 4, C. The upright hyphae have now converged upon the common point *b*. At *d* is the edge of the next spermogonium. The growth of the fungus between spermogonia has not kept pace with this, so there are open spaces between the hyphae at *a* and *c*.

Soon after this the different kinds of cells within the spermogonium become differentiated. In Plate 5, A, the slender, tapering hyphae just beneath the epidermis and nearly parallel to it at *a* and *d* will become the paraphyses. The heavy central upright hyphae at *b*, with coarsely alveolar cytoplasm and blunt tips pressed against the epidermis, are the buffer cells, whose main function is to lift the epidermis and withstand its pressure. Growing in between the buffer cells at the base are slender pointed cells at *c* and *e*. These are the first of the spermatiophores.

The epidermis above the spermogonium is strained, and its cells are somewhat flattened. The lifting of the epidermis at the spermogonia also raises it, to a lesser extent, between them. The few hyphae of the subepidermal mat between the spermogonia (pl. 5, A, *f*) are often displaced and broken by the lifting of the epidermis.

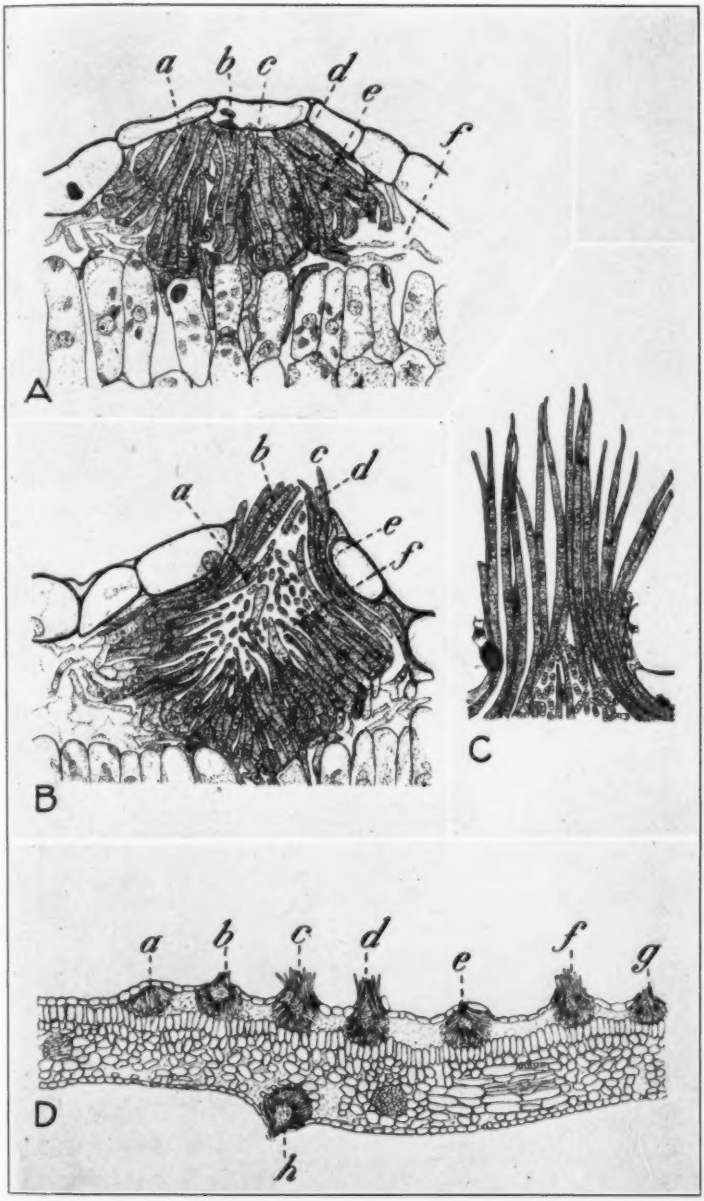
EXPLANATORY LEGEND FOR PLATE 5

A.—Spermogonium from 6-day infection: *a* and *d*, Young paraphyses; *b*, buffer cells; *c* and *e*, spermatiophores; *f*, broken, dying hyphae. × 640.

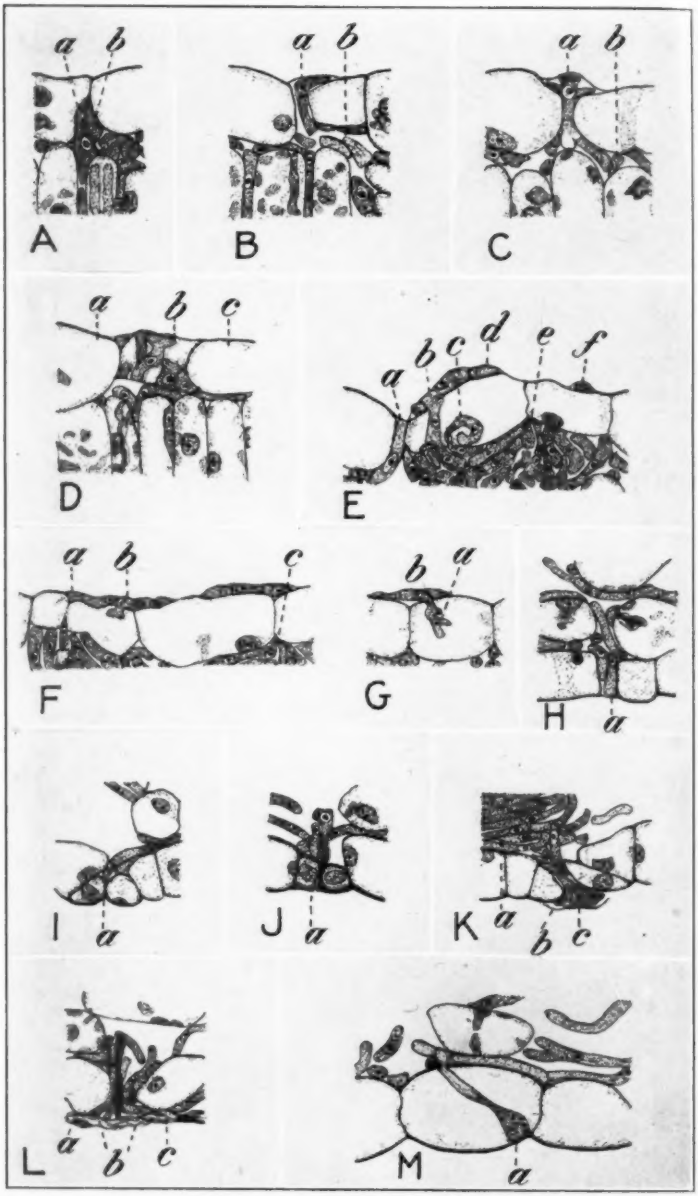
B.—Six-day infection; spermogonium just opening: *b* and *c*, Paraphyses; *a* and *f*, buffer cells; *e*, spermatia. × 640.

C.—Seven-day infection: Tuft of paraphyses extruded from ostiole of spermogonium. × 640.

D.—Semidiagrammatic drawing of median section of 6-day infection: *a* to *g*, Subepidermal weft between palisade and epidermis, bearing spermogonia at *a*, *b*, *c*, *d*, *e*, *f*, and *g*; smaller stroma and spermogonium (*h*) at lower epidermis. × 115.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

The spermatiophores (pl. 5, B) now increase rapidly in numbers. Cells along the base and sides of the spermogonium branch and grow in toward the center. The interpolation of new spermatiophores among the old enlarges the spermogonium and rounds it out at base and sides. As it arches outward a central cavity forms (pl. 5, B, *e*) and into this the spermatiophores set free the spermatia. The buffer cells, which earlier formed the central columns, braced against the epidermis (pl. 5, A, *b*), are now separated from each other by the spermatiophores (pl. 5, B, *a*, *f*) and can be seen here and there jutting into the central cavity. The paraphyses in the meantime have converged and turned outward (pl. 5, B, *b*, *c*), breaking through the epidermis and forming the ostiole. These paraphyses (pl. 5, C) elongate into a brush of straight tapering hyphae. So far as noted, the extruded part of a paraphysis is nonseptate and contains one nucleus or, more commonly, two nuclei.

Some idea of the appearance of the infection as a whole at this stage may be obtained from the diagram of a central section through a well-developed 6-day infection in Plate 5, D. There are spermogonia at different stages of development on the stroma at *a*, *b*, *c*, *d*, *e*, *f*, and *g*. The areas beneath the epidermis between spermogonia appear almost empty. The few hyphae of the subepidermal stroma between the spermogonia are broken, and often dead and nearly transparent. A little later, however, this space is filled in by a secondary growth of hyphae.

Usually a similar but smaller stroma, which bears a few spermogonia, forms at the lower surface of the leaf. Plate 5, D, *h*, shows the beginning of such a stroma. A count of 100 spermogonia in 6-day infections shows that 73 open upon the upper surface of the leaf and 27 on the lower. A similar count in 10-day infections gives 77 on the upper surface and 23 on the lower.

Spermogonia and receptive hyphae usually develop at the same time. In Plate 4, A, already referred to, spermogonia are forming at *b* and *e*, and receptive hyphae are pushing out between the cells of the upper epidermis at *c* and *d*. In Plate 6, A, two hyphae (*a*, *b*) have split the epidermal walls apart for two-thirds of the distance to the leaf surface. In Plate 6, E, the hypha (*a*) has reached the surface, and its tip is flattened against the cuticle of the leaf.

The first receptive hyphae antedate the aecia by several days and may even antedate the spermogonia, as in Plate 6, B and C. In

EXPLANATORY LEGEND FOR PLATE 6

A.—Detail of 6-day infection: *a* and *b*, Two hyphae pushing up between cells of the upper epidermis.
B.—Six-day infection: *a*, Hypha grown out between epidermal cells and on a short distance beneath the cuticle; *b*, subepidermal mycelium.
C.—Six-day infection: *a*, Hypha grown out between cells of the upper epidermis, with three divergent branches; *b*, subepidermal mycelium.
D.—Six-day infection: *a* to *c*, Detail of upper epidermis; *b*, hyphae growing toward surface of leaf.
E.—Detail of 6-day infection: *a*, Intercellular receptive hypha; *c*, haustorium; *e*, young spermogonium from which an intracellular hypha (*b*) grew up and gave rise to the subcuticular hypha *d*; *f*, cross section of subcuticular hypha.
F.—Six-day infection: *a*, Subcuticular growth from which a haustorium is forming at *b*; *c*, young spermogonium beneath the epidermis.
G.—Detail of upper epidermis in 6-day infection: *b*, Subcuticular hypha, from which a haustorium has formed at *a*.
H.—Detail of 7-day infection: *a*, Hyphae between cells of the lower epidermis.
I.—Detail of 7-day infection: *a*, Hypha wedged into the inner angle of a shut stoma.
J.—Detail of 7-day infection: *a*, Hypha wedged into the inner angle of a shut stoma.
K.—Detail of 7-day infection: *a*, Paraphyses; *b*, membrane; *c*, broad-top emergent hypha in stoma (cut obliquely) adjoining a half-grown spermogonium.
L.—Detail of 7-day infection: *a* and *c*, Dead cells of lower epidermis; *b*, hyphae crowding down against lower epidermis.
M.—Eight-day infection: *a*, Intracellular receptive hypha at the lower epidermis. (All ×640.)

Plate 6, B, a hypha (*a*) has grown up between the epidermal cells and after reaching the outer epidermal wall has grown farther, parallel to the leaf surface, underneath the cuticle. These hyphae seem to be unable either to pierce the cuticle mechanically or to dissolve it chemically but are able to effect a separation between the cuticle and the inner layers of the epidermal wall and to grow for considerable distances beneath the cuticle. Sometimes a hypha emerging upon the surface gives rise to a single subcuticular hypha, sometimes to several. In Plate 6, C, the hypha at *a* is forming three young branches, which have started off in different directions under the cuticle.

The infection from which Plate 6, B and C, were drawn is small. It is in a slightly earlier stage of development than the one shown in Plate 4, A. The subepidermal mat of hyphae is still thin (pl. 6, B, *b*; C, *b*) and without signs of spermogonia. The mycelium has not yet reached the lower epidermis of the leaf, and it will be several days before the first aecium is initiated. There is no possibility here that these emergent hyphae originated in an aecial primordium.

Emergent hyphae usually grow up between epidermal cells, but there is some evidence that they can also push up through the host cell. In Plate 6, D, is a case open to two interpretations. It may be that the hyphae at *b* are seen in flat view as they push up between epidermal walls lying in the plane of the section. It is also possible that they are growing up intracellularly. Certainly the host cell (*b*) has lost turgidity, for the side walls of the adjoining epidermal cells (*a*, *c*) are bulging into it.

In Plate 6, E, the evidence is clearer. The hypha (*b*) grew straight up through the epidermal cell and then grew on as a subcuticular hypha (*d*). The habit of growth of the receptive hypha (*b*) is in marked contrast to that of the haustorium (*c*) alongside it. Subcuticular hyphae grow in a cramped space and are more or less flattened by pressure, as may be seen from the cross section of such a hypha. (Pl. 6, E, *f*.) In this instance receptive hyphae and spermogonia are closely associated, for there is a young spermogonium below the epidermis at *e*.

These subcuticular hyphae, like the mycelium producing them, are septate and haploid. Also, like the mycelium below, they have not lost the ability to produce haustoria. When a hypha has grown subcuticularly for some distance, its growing tip is remote from the base of supplies. At *b*, in Plate 6, F, a young haustorium is forming from an outer hypha and in Plate 6, G, at *a*, is a full-grown haustorium, which entered from the outer face of the epidermal cell. So far as appearances go, these subcuticular hyphae differ from vegetative hyphae only in their location.

When the spermogonia are young and the subepidermal mycelium is vigorous, emergent hyphae with subcuticular extensions are formed in abundance. A little later, as the spermogonia mature, lifting the upper epidermis as they grow, the subepidermal hyphae between spermogonia become broken and almost disappear. If the connections to the surface hyphae are broken they, too, shrivel and disappear. This subepidermal space is soon filled in by a secondary growth of mycelium, however, and new emergent hyphae form similar to the first lot.

Meanwhile, the vegetative mycelium of the young growing infection is spreading in all directions and soon reaches the lower epidermis of

the host leaf. Here a new series of receptive hyphae is formed, some pushing into stomatal apertures (pl. 6, I, J, K), others squeezing between epidermal cells (pl. 6, H), and still others growing through an epidermal cell (pl. 6, M).

Plate 6, H, drawn from a 7-day infection, shows a bit of the lower epidermis between two cells of which, at *a*, hyphae have grown. One has reached the lower surface of the leaf but is still inclosed by the cuticle. These receptive hyphae and the mycelium above them are haploid.

Hyphae are to be found in stomatal apertures but are not nearly so abundant as in *Puccinia triticina*. In Plate 6, I and J, the stoma is closed, but the hyphae above it are definitely aimed at the small crack between the guard cells. In Plate 6, K, in which the stoma is cut obliquely, the hypha has been thrust through the stomatal opening (pl. 6, K, *c*) and has broadened out at the tip. A sort of thin film or membrane (pl. 6, K, *b*) covers the exposed tip of the hypha. This has frequently been observed in stomatal hyphae and may represent a substance secreted by the hypha.

This emergent hypha (pl. 6, K, *c*) has formed next to a half-grown spermogonium, and the hypha itself has grown out of the group of young paraphyses (pl. 6, K, *a*) of the spermogonium. This is of fairly frequent occurrence and would seem to indicate that there is no potential difference between a paraphysis and a receptive hypha. So far as can be determined, the same hypha can develop into either structure.

In Plate 6, L, a minor injury killed two or three epidermal cells (pl. 6, L, *a*, *c*), and no less than five hyphae (pl. 6, L, *b*) have grown down to make contact with the disorganized cells. Perhaps the outer air, filtering through the dead cells, served as the stimulus inciting this growth.

In Plate 6, M (drawn at higher magnification than the preceding), a hypha has grown down through a cell of the lower epidermis and its tip has flattened against the outer epidermal wall. There are two small nuclei (pl. 6, M, *a*) near the tip. Hyphae of this sort also are regarded as receptive hyphae.

GREENHOUSE DATA ON HETEROTHALLISM

Although infections of *Puccinia coronata* may grow faster or slower according to local conditions, the general course of development is similar in all, up to the maturity of the spermogonia. From then on there is a difference. Some of the mycelia produce fertile aecia. Others remain sterile, i. e., produce no aeciospores.

An attempt was made to determine whether this difference is due to heterothallism. Infections are classified as single (not in contact with others), double (two growing in contact), and multiple (several infections confluent). In a heterothallic rust with two sexual strains, if the infections are left undisturbed and spermatia are not transferred from one infection to another by outside agencies, it would be expected that all the single infections and 50 per cent of the double infections would remain sterile, while the other 50 per cent of the double and nearly all the multiple infections would produce aeciospores.

In a preliminary test, a much higher percentage of the infections developed aecia than would be expected. The inoculated plants were covered with tarlatan cages, but it was discovered that minute yellow insects not excluded by the cages were going from one infec-

tion to another, feeding on the spermogonial exudate and incidentally transferring spermatia from one infection to another. As a result, out of 248 single infections, 37 per cent had developed open aecia by the twentieth day after inoculation, 71 per cent by the thirtieth day, and 83 per cent a week or so later. All the multiples bore open aecia by the twentieth day. The effectiveness of insect transfer, at least, is amply demonstrated.

In a second experiment some of the inoculated plants bore only one infection, and from others all but one were removed. These plants were carefully isolated. The results are tabulated separately. (Table 1.) Nearly all the material was fixed between the thirtieth and the fiftieth day. Comparatively little of it reached the age of 50 days.

TABLE 1.—*Percentage of infections of Puccinia coronata with open aecia at different ages*

[1930 material]

Group	Infections of indicated age									Infections with open aecia expected
	20 days			30 days			30 to 50 days			
	Total	With open aecia		Total	With open aecia		Total	With open aecia		
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*
Singles.—One infection on a plant	8	0	0	8	1	12	8	1	12	0
Singles.—Several infections on a plant	79	8	10	[a] 65	16	25	63	18	29	0
Doubles	6	3	50	[b] 5	3	60	5	3	60	50
Multiples	3	1	33	3	3	100	3	3	100	(c)

[a] 2 sterile infections died, and several others were fixed.
[b] 1 fertile infection was fixed.
[c] Nearly all.

There is evidence here of heterothallism. Eight of the Rhamnus plants bore one infection each. Of these eight infections, only one developed aecia, and it may be that, in spite of all attempts to isolate it, accidental fertilization occurred here. Where several singles occurred on one plant the chances that spermatia might be transferred by insects are porportionally increased, and this is reflected in the ratios. The number of doubles and multiples recorded in Table 1 is too small to be significant, but the evidence, so far as it goes, supports the assumption that *Puccinia coronata* is heterothallic.

On a plant bearing six infections well separated from one another, the spermatia were thoroughly mixed on the seventh day. Five of

EXPLANATORY LEGEND FOR PLATE 7

A.—Portion of unfertilized aecium from 10-day infection showing the growth of hyphae to the stoma at *a*. ×640.

B.—Portion of unfertilized aecium from 10-day infection showing longitudinal section of stoma at *a*, and the hyphae above centered upon it. ×640.

C.—Detail from 8-day unfertilized infection: *a*, Probably remnant of subcuticular growth; *b*, decadent intracellular hypha from subepidermal hypha (*c*). ×1,020.

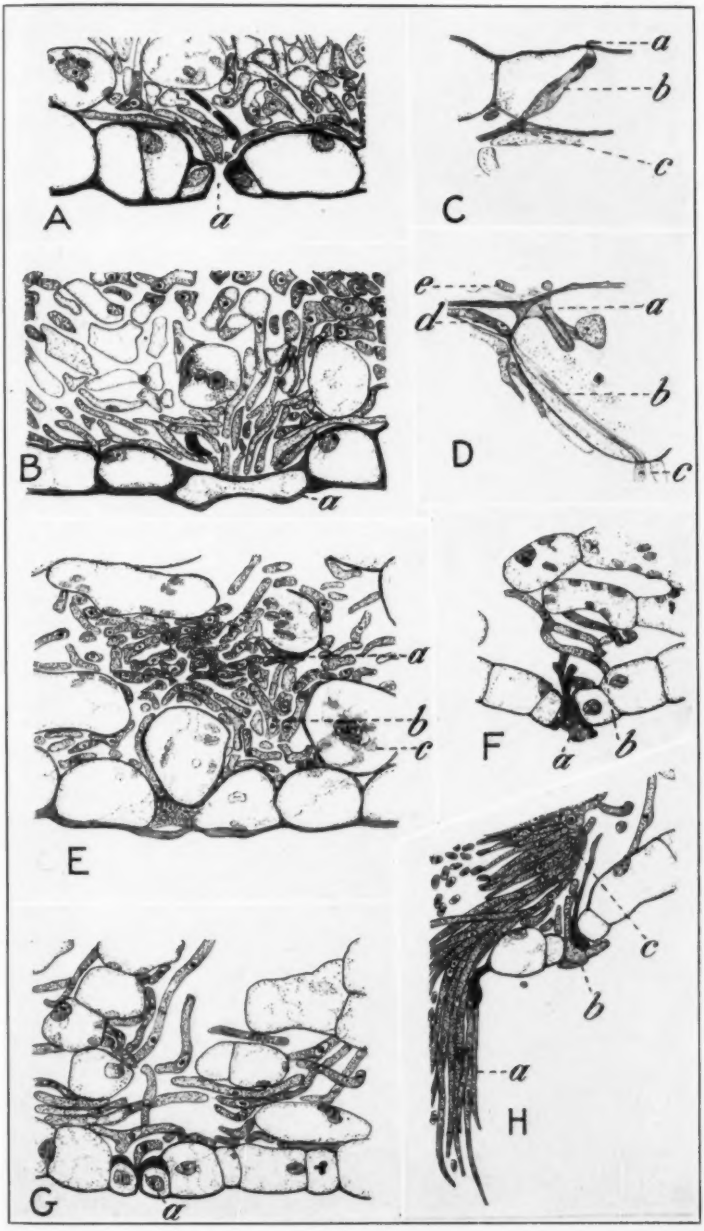
D.—Bit of upper epidermis of 8-day sterile infection near spermogonium: *a*, Heavily insheathed remnant of a haustorium; *c*, subepidermal hypha that grew through the epidermal cell (*b*) and on to form a hypha (*d*); *e*, spermatium. ×1,020.

E.—Young sterile aecium: *a*, Upper half, of small dense cells; *b*, lower half, of larger cells; *c*, dead host cell. ×640.

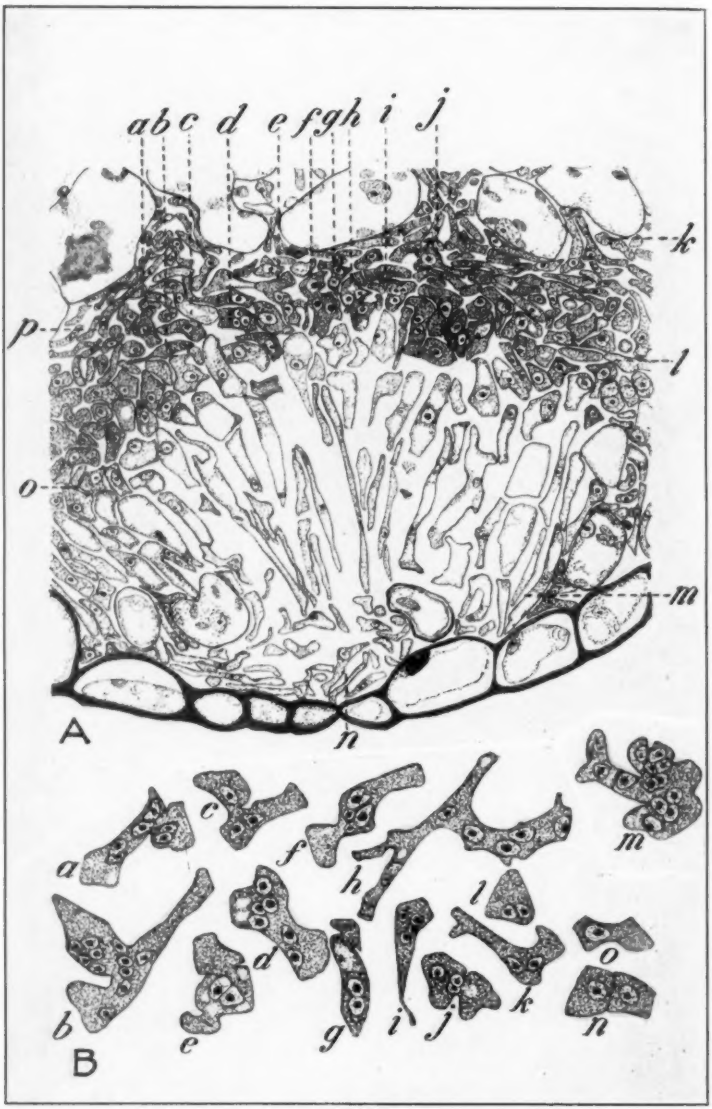
F.—Eight-day sterile infection: *a*, Stoma filled with receptive hyphae from vegetative mycelium (*b*). ×640.

G.—Beginning of aecium in 8-day infection: *a*, Young receptive hypha at stoma. ×640.

H.—Portion of spermogonium at lower surface of leaf in 8-day sterile infection: Paraphyses (*a*) and receptive hyphae in stoma (*b*) have grown from the margin of spermogonium at *c*. ×640.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

the six infections produced fertile aecia. This fact, combined with the fact that seven out of eight of the best isolated infections bore no aeciospores, affords strong evidence that spermatia of *Puccinia coronata* function in initiating the sporophyte generation. The results as a whole show clearly the agency of insects in transferring the spermatia.

THE UNFERTILIZED INFECTION

In the isolated infection the vegetative mycelium, so far as noted, is composed of uninucleate cells. Aecia form regularly, develop up to a certain stage, and then deteriorate.

The sterile aecium (pl. 7, G, drawn from an 8-day infection) begins as a small cluster of hyphae in the large intercellular spaces of the spongy mesophyll near the lower epidermis. At first it is distinguishable from the general mycelium only by the closer grouping and denser contents of the hyphae. In *Puccinia coronata* the aecial primordium is not necessarily initiated about the base of a receptive hypha at a stoma. The aecium sometimes comes first and the receptive hyphae later. In Plate 7, G, the aecium is well begun, but the only indication of receptive hyphae is the young hypha (*a*) in the inner angle between the two guard cells of a shut stoma. There is no fully formed receptive hypha connected with this young aecium.

Although an aecium may be well started without having any receptive hypha directly connected with it, there is usually an abundance of hyphae not far away. In the 8-day infection from which Plate 7, G, is drawn there are stomata filled with hyphae. (Pl. 7, F, H.) In Plate 7, F, three or four hyphae in turn have forced themselves into the stoma at *a*. These receptive hyphae took their origin in ordinary vegetative hyphae above, at *b*. In Plate 7, H, the occupied stoma lies close to the ostiole of a spermogonium. The paraphyses at *a* and the receptive hyphae at *b* have a common origin in cells of the side wall of the spermogonium near *c*. In both cases (pl. 7, F, H) the first hyphae to enter the stoma have died, and only the latest entrants are still living.

By further growth and branching within the area of the young aecium, the hyphae form a dense little nest. Cells within the lower half of this nest soon begin to expand. An early stage of this differentiation of the young aecium into upper and lower halves is represented in Plate 7, E. At *a*, the tightly snarled hyphae are small and dense and closely septate. Lower, at *b*, the cells are somewhat larger and their contents more vacuolate.

Even at this stage in the development of the aecium (pl. 7, E) there may be no surface hyphae directly connected with it. A careful examination of the sections from beginning to end of this young aecium reveals no receptive hyphae in stomata, between epidermal cells, or growing out intracellularly through epidermal cells. The only hyphae that could possibly be considered receptive are some that have grown down to make contact with a dead, shrunken epidermal cell in an adjoining section.

EXPLANATORY LEGEND FOR PLATE 8

A.—Median section through aecium of 10-day sterile infection: *a* to *j*, Numerous cells, each with two or three nuclei in the upper arch of denser cells (*k*, *l*, *o*, *p*); the open space-making layers below (*l*, *m*, *n*, *o*) are uninucleate. ×640.

B.—Cells from aecia of a sterile 10-day infection: *a* to *m*, Representative multinucleate cells; *n* and *o*, typical uninucleate cells. ×1,020.

It may be that air, entering the leaf from without, whether it enters through a dead cell or through a stoma, exerts an attractive influence on the fungus. Hyphae will grow to a stoma whether that stoma adjoins a spermogonium, ordinary mycelium, or an aecium. Plate 7, A and B, show hyphae growing to stomata adjoining sterile aecia. In Plate 7, A, *a*, the stoma is shown in cross section, and in B, at *a*, the stoma is cut longitudinally. In neither is the stoma opposite the center of the aecium. In both, hyphae have grown in from all directions toward the stomatal aperture but have not actually entered it.

Reference was made earlier (pl. 6, E, M) to intracellular hyphae that push out through an epidermal cell and sometimes pierce the outer epidermal wall and grow on subcuticularly. When they fail to make contact with spermatia, these receptive hyphae, like those in the stomata, soon die. In Plate 7, C, the subepidermal hypha (*c*) gave rise to the intracellular hypha (*b*), which grew through the epidermal cell to its outer wall. At *a* is a minute object which may be the dried remnant of a former subcuticular hypha. The intracellular growth is dead. Plate 7, D, shows the dead intracellular hypha (*b*) coming from the mycelial hypha (*c*). This epidermal cell lies at the edge of a spermogonium, and the hypha (*b*) issued into an adjoining crushed, empty epidermal cell at *d*. At *a* is a dead and heavily insheathed structure, apparently the remnant of a haustorium entering the cell from earlier subcuticular growth.

As the haploid mycelium spreads through the host leaf, new aecia form in succession in the younger marginal growth. A section through the center of a well-grown, 10-day, unfertilized infection shows all stages in the development of aecia from the early stages in the margin to full-grown and degenerating sterile aecia in the center. The young haploid aecium shown in Plate 7, E, is found near the edge of such an infection; the larger sterile aecium shown in Plate 8, A, occurs farther in; while at the center of the same infection are full-grown aecia showing degenerative changes characteristic of older aecia when fertilization fails to take place. No indication of fertilization was noted at any point in the infection.

In the aecium shown in Plate 8, A, referred to above, the differentiation into upper and lower areas is completed. The lower area of vacuolated cells in the aecium has expanded into the shape of a very thick, biconvex lens. (Pl. 8, A, *l–m*.) The cells of this looser or "space-making" tissue (pl. 8, A) are no longer increasing in number but are elongating radially, presenting in section a fan-shaped arrangement centered at *n*. These cells are still uninucleate and loosely spaced; many are empty and quite transparent. The area of denser cells (pl. 8, A, *k*, *l*, *o*, *p*) that caps the space-making tissue is composed of five or six layers of short, thick, often quite irregular cells. The majority are uninucleate, but in the central region (pl. 8, A, *a–j*) there are a number of cells containing two or three nuclei. As stated above, this aecium is quite certainly unfertilized, for all the older aecia of the same infection (Table 2, 10-day) show marked evidence of degeneration, yet it contains several dozen binucleate or trinucleate cells.

Ordinarily, from the tenth day on, multinucleate cells are regularly present in sterile aecia. It has been assumed hitherto that the presence in an aecium of cells containing more than one nucleus means sporophytic growth which will lead to spore formation. The

finding of multinucleate cells in nearly 100 per cent of the older sterile aecia of *Puccinia coronata* is a point of theoretic interest, and a detailed study has been made of these sterile infections of different ages.

The question arises as to the mode of origin of these binucleate and trinucleate cells. Some of them (pl. 8, A, *e, f, h*) are highly irregular in form. Their unexpected angles, clefts, and branches may be the result of either the fusion of two or more cells or the irregular growth of one. Others (pl. 8, A, *b, c*) are simple in form and give no suggestion from their shape that they are the product of recent fusion.

Plate 8, B, *a* to *m*, shows, at high magnification, representative multinucleate cells from sterile aecia of this and slightly later stages, with a few typical uninucleate cells (*n, o*) for comparison.

No great insight as to the mode of origin is to be gained by a study of these cells. In some cases an origin by fusion seems possible, for in spite of the growth that the cells may have made since fusion, they still suggest by their shape a double origin. Where there is a relatively narrow isthmus between two parts of a cell (pl. 8, B, *a, b, c, e, f, g, j*) there is a possibility of origin by fusion. Even here, however, there is the alternative possibility of the irregular growth of a single cell constrained into irregular forms by the pressure of adjoining cells. One point seems clear—it can not be assumed that multinucleate cells are the result of multiple fusion. Cells (pl. 8, B, *b, m*) that contain 10 or 12 nuclei more probably have arisen by simple growth and nuclear divisions or possibly by a single initial fusion followed by such growth.

Soon after the stage of development represented in Plate 8, A, sterile aecia begin to degenerate. Plate 9, A, shows a semidiagrammatic section through a sterile 16-day infection. The leaf is considerably hypertrophied, as may be seen by comparing its thickness at the center and at the ends of the drawing; the whole infected area is arched upward, making a raised spot on the upper surface of the leaf.

The younger aecia of the series, located either in the fresh young marginal growth (pl. 9, A, *a, f*) or in a belated development on the upper surface (pl. 9, A, *g, h*) are vigorous in appearance and resemble closely the aecium shown in Plate 8, A. The older aecia (pl. 9, A, *b, c, d, e*) are showing signs of degeneration.

The cells of these older sterile aecia are somewhat impoverished and take a diffuse stain, although comparatively few of the aecia in this case contain dead cells. At this stage each of the larger aecia has pushed the lower epidermis outward into a convex curve. (Pl. 9, A, *b, d.*) Later, the sterile aecium shrinks and may even be marked by a depression of the surface.

A summary of the condition of aecia in sterile infections of *Puccinia coronata* of different ages has been made. In this study (Table 2) the aecia of each infection are classified as (1) too young to show multinucleate cells, (2) containing living multinucleate cells, (3) sterile and containing both living and dead multinucleate cells, and (4), in a few cases, cells with two to four nuclei and spores. Counts were made in infections of different ages and the data tabulated. Each horizontal line (Table 2) represents the aecia of one infection.

TABLE 2.—*Summary of condition of aecia in sterile infections of Puccinia coronata of different ages*

[1928 material]

Age of infection (days)	Number of aecia showing indicated condition [a]				Age of infection (days)	Number of aecia showing indicated condition [a]			
	Too young to show multinucleate cells	Containing multinucleate cells living	Containing both living and dead multinucleate cells	Containing multinucleate cells and spores		Too yount to show multinucleate cells	Containing multinucleate cells living	Containing both living and dead multinucleate cells	Containing multinucleate cells and spores
10	14	13	9	.....	16	5	13	8	.....
11	7	8	.....	.....	16	30	27	.....	.....
11	3	20	.....	.....	17	10	4	.....	.....
12	4	4	.....	.....	17	13	19	32	.....
12	8	.....	.....	.....	17	5	18	19	.....
13	10	4	.....	1	18	3	11	5	1
13	23	.....	.....	.....	18	.....	11	4	.....
13	15	10	.....	.....	23	1	20	18	.....
13	16	.....	.....	1	26	1	6	6	.....
14	1	4	.....	.....	26	2	6	15	.....
14	25	2	.....	.....	26	13	15	.....	.....
14	10	3	.....	.....	28	.....	16	8	.....
14	9	4	.....	.....	30	.....	1	35	.....
15	8	.....	.....	.....	30	.....	.....	41	.....
15	1	.....	3	.....	62	.....	.....	20	.....
16	17	2	.....	.....	62	.....	.....	12	.....
16	2	18	10	.....					

[a] Leaders indicate that aecia were not found.

From Table 2 it will be seen that the aecia of a single infection present a broad range of development, for during a considerable period new aecia keep forming in the fresh marginal growth of the infection. There is also considerable variation between infections of the same age. This is due partly to differences in the environment, for infections grown late in the spring and thus exposed to higher temperatures and stronger light develop faster than do those of early spring. The variation may also be due to localized differences within the host tissues, for of two infections of the same age growing side by side on the same leaf, one may develop faster than the other.

In three of the infections included in the study (Table 2) a single aecium developed spores. In such cases, when the infection is young enough, the sporophyte generation can spread farther. In greenhouse studies, however, it is not rare to find an old decadent infection with a single open aecium. This is perhaps explained by the fact that the transfer of spermatia by insects has taken place so late that only one aecium is still young enough to respond.

In general, up to the fifteenth day (Table 2) over half of the aecia are too young to have developed multinucleate cells, and where they do occur these multinucleate cells are still living. Beginning with infections 16 days old, the proportion of young aecia decreases and the proportion with living multinucleate cells increases. In the later

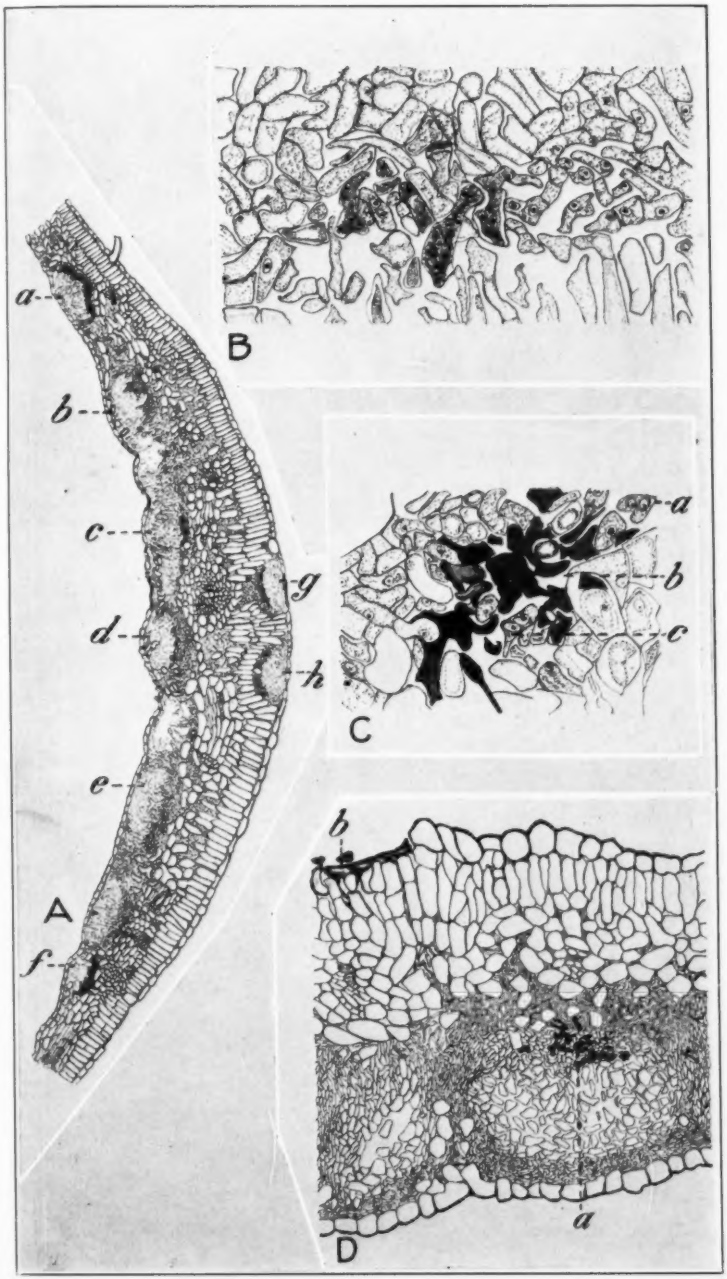
EXPLANATORY LEGEND FOR PLATE 9

A.—Diagram of section through a 16-day sterile infection: *a*, *f*, *g*, and *h*, Younger aecia; *b*, *c*, *d*, and *e*, older aecia. ×45.

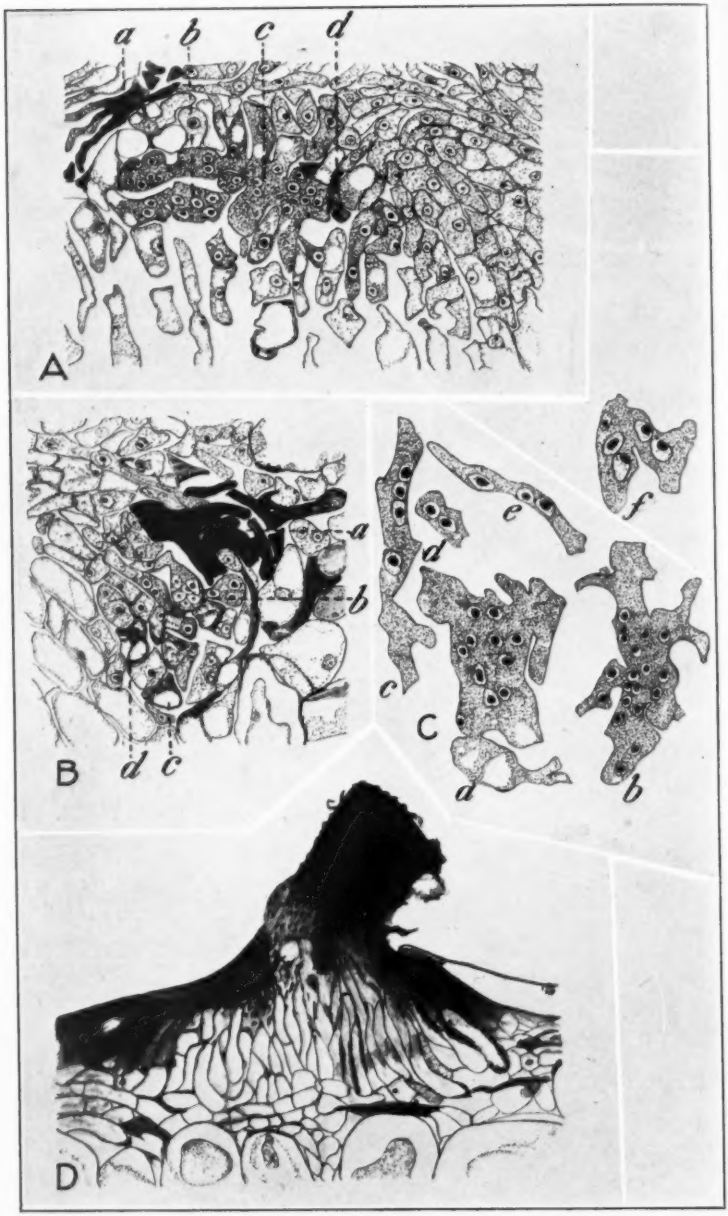
B.—Detail from upper central part of aecium from 23-day sterile infection, showing deteriorating multinucleate cells. ×640.

C.—Twenty-three-day sterile infection: *b*, Dead multinucleate cells; *a* and *c*, living multinucleate cells. ×640.

D.—Diagram of sterile aecium of 26-day infection: *a*, Dead multinucleate cells; *b*, denuded leaf surface. ×115.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

history of the sterile infections (Table 2, 18-day to 62-day) there is a steady decrease in the number of young aecia and later a decrease in the number of those whose multinucleate cells are all living, until finally all the aecia are in the third class, i. e., they contain both living and dead multinucleate cells.

A similar study was made of sterile infections in the rust grown in 1930. Here, as before, multinucleate cells were of regular occurrence in older sterile aecia, but in these slower-growing infections the multinucleate cells did not appear before the twenty-fifth day after inoculation, fully two weeks later than in the Canadian rust used in 1928. Once formed, however, the multinucleate cells followed the same course of slow growth, nuclear multiplication, and subsequent deterioration. A few multinucleate cells were still living in an infection 49 days old.

Plate 9 shows details of these degenerative changes within the sterile aecium. A group of multinucleate cells (pl. 9, B) shows the beginning of degeneration in a 23-day infection. The number of nuclei in a cell varies from two to eight. Degeneration is evidenced by the dimness of detail in these cells and a decided tendency to stain red with safranine. As the cells around this group are impoverished in content and stain very faintly, the contrast between the two is sharp and can be seen clearly even with the low power of the microscope.

In this same infection are other aecia in all stages of degeneration. In Plate 9, C, nearly all the multinucleate cells are dead and densely stained with red. Only two binucleate cells (*a*, *c*) are left alive.

Something of the appearance of the sterile aecium a little later (after 26 days) is shown in Plate 9, D. Both the fungus and its host have increased greatly in size. In the upper half of the aecium at *a* is a group of dark-stained dead multinucleate cells. Multinucleate cells, living or dead, are ordinarily located in the upper central part of the aecium (pl. 9, D, *a*), but in older aecia they are sometimes separated into patches and may be erratically placed near the side walls of the aecium, or even out in the space-making tissue. It is probable that they all originate as figured in the younger aecia (pl. 8, A) but become displaced by subsequent localized growth in adjoining areas of the aecium.

Among the dead multinucleate cells are usually a few survivors, which may grow further. In Plate 10, A, between the dead or dying cells at *a* and *d* are several large living multinucleate cells. One of them (*c*) is irregularly lobed and branched; another (*b*) suggests by its shape that it may be a product of fairly recent fusion. In Plate 10, B, is another group, also from a 26-day infection. Beside a group of dead cells (*a*) is a nest of living multinucleate cells (*b*). Some of these cells look vigorous, but others (*c*, *d*) are dying.

Sometimes one of these sterile infections, if very favorably placed, may live on for eight or nine weeks. Within its aecia are still to be found a few living multinucleate cells. Plate 10, C, shows such cells from a 62-day infection. Some are small and contain few nucle

EXPLANATORY LEGEND FOR PLATE 10

A.—Multinucleate cells in aecium of 26-day sterile infection: *a* and *d*, Dead or dying cells; *b* and *c*, large living cells. ×640.

B.—As in A: *a*, Dead cells; *c* and *d*, dying cells; *b*, living cells. ×640.

C.—Living multinucleate cells (*a*, *b*, *c*, *d*, *e*, *f*) from an aecium in a sterile 62-day infection. ×1,020

D.—Dead spermogonium from a 23-day sterile infection. ×640.

(pl. 10, C, *c*, *d*, *e*, *f*), others have grown and branched irregularly (pl. 10, C, *a*, *b*) and may contain as many as 20 nuclei. The age and mode of origin of cells like these are matters of conjecture only.

The spermogonial exudate in *Puccinia coronata* is never abundant, and the spermogonia for the most part are short lived. The spermogonia in these sterile infections do not, as in *P. graminis*, continue to function throughout the lifetime of the fungus. They soon die. In one 23-day infection no functioning spermogonium remains. Plate 10, D, shows the condition of the spermogonia. It has already been noted that the spermogonia form on a continuous cushion or stroma beneath the epidermis. Before the sterile infection is very old, sometimes as early as the fifteenth day, this stroma begins to be sloughed off, leaving the palisade of the leaf tissue exposed. The edge of one of these denuded areas is shown in Plate 9, D, at *b*.

FERTILIZATION AND THE FERTILE AECIUM

A monosporidial infection bears both spermogonia and receptive hyphae, but the spermatia borne by an individual apparently can not start the sporophyte generation in that same mycelium, for when an infection is carefully isolated it remains sterile and produces no aeciospores. But when spermatia are transferred to infections of opposite sex, the sporophyte generation is initiated and fertile aecia form.

When spermatia are transferred either by insects or by laboratory methods they are applied to the leaf surface (either upper or lower) at the infected area. Spermatia scattered along the surface of the epidermis can enter at any point where the mycelium reaches the surface.

Plate 11, C, shows, at low magnification, part of a section through a 12-day infection. At *c* is one spermogonium and at *a* a lateral section through a second. There are sporophytic cells between the two at *b*; this area is shown enlarged in Plate 11, A.

In Plate 11, A, a hypha (*e*) of the subepidermal mycelium grew into an epidermal cell at *d*, and out through the outer wall to form a subcuticular hypha (*c*). The connection between *c* and *d* is found in the next section. At *a*, on the surface of the leaf, are spermatia. At *b*, where hypha and spermatia meet, there is a break in the cuticle and three small dark masses (possibly of spermatial origin) are embedded in the end of the hypha. That spermatial nuclei entered the hypha and passed down into the inner mycelium seems fairly certain, for binucleate cells are seen in the subepidermal region at *f*, *g*, and *h*. Both the intracellular hypha and the subcuticular hypha are now decadent. At *i*, within the body of the spermogonium, are two binucleate cells. It does not seem probable that sporophytic hyphae originating outside a mature spermogonium would later grow into it.

EXPLANATORY LEGEND FOR PLATE 11

A.—Detail from C enlarged: *a*, Spermatia; *b* and *c*, subcuticular hyphae; *d*, intracellular hypha from subepidermal hypha (*e*); *f*, *g*, *h*, and *i*, sporophytic cells. × 640.

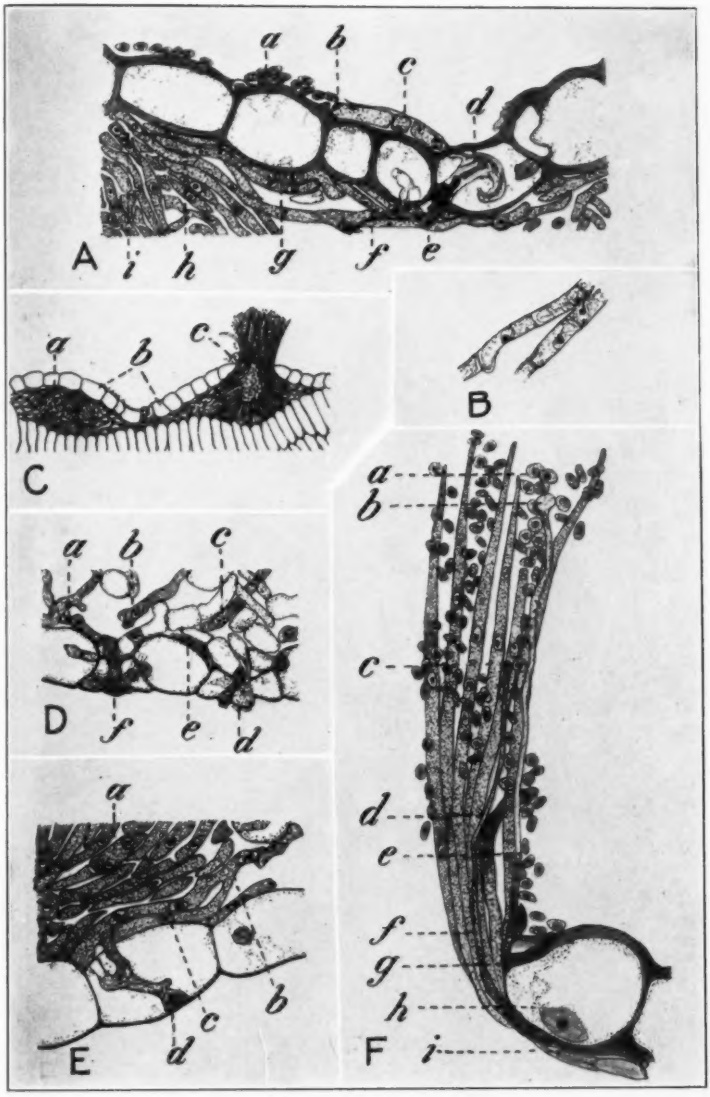
B.—Detail of mycelium of 13-day infection showing two hyphae tangent and fused at the point of tangency. × 1,020.

C.—Diagram of subepidermal weft of 12-day infection: *a* and *c*, Spermogonia; *b*, detail enlarged in A. × 115.

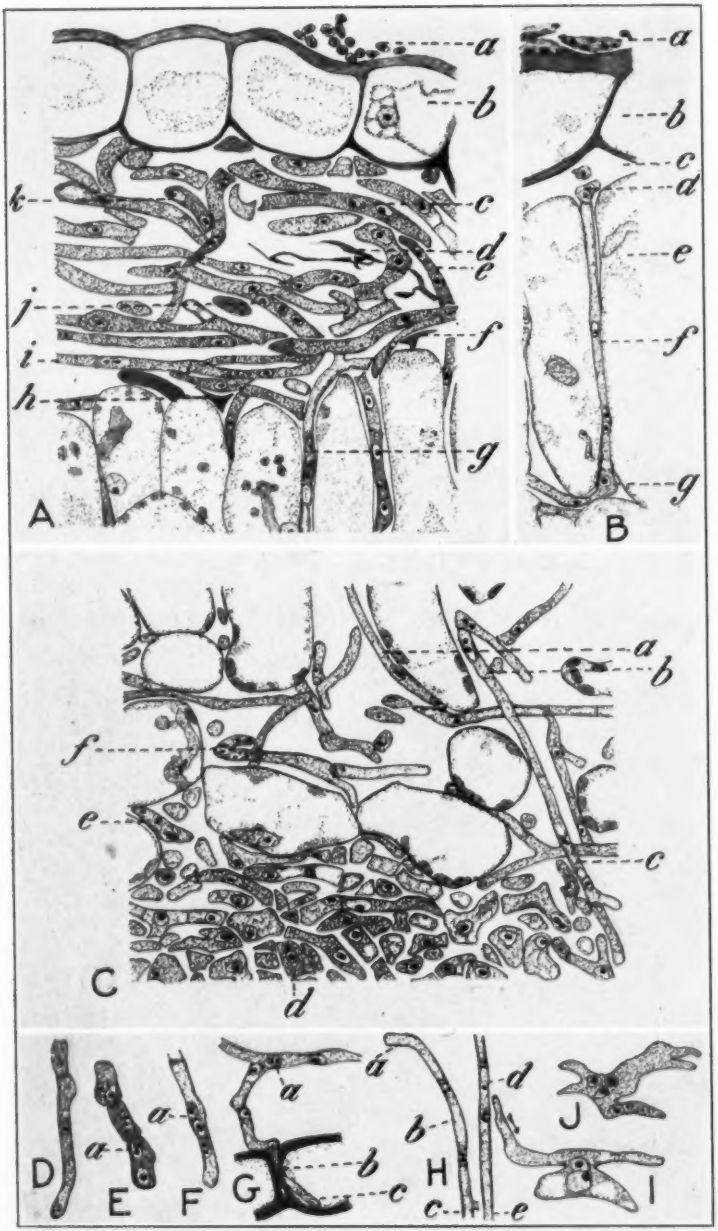
D.—Detail from lower leaf surface of 8-day infection: *d* and *f*, Stomatal hyphae; *a*, *b*, *c*, and *e*, sporophytic hyphae above. × 640.

E.—Seven-day infection: *a*, Part of spermogonium; *b* and *c*, young paraphysis giving rise to an intracellular branch (*d*) that has grown out through the epidermal cell; the branch contains six nuclei. × 1,020.

F.—Portion of tuft of paraphyses of a spermogonium, with adherent spermatia, from 12-day infection: *a*, Expanded spermatia, *b*, germinating spermatium, with germ tube leading to *e* and *g*; *c*, second germ tube, leading to *f* and *i*; *d*, decadent paraphysis; *h*, epidermal cell. × 1,020.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

It is possible that spermatial nuclei entered at a second point somewhere within the spermogonial area.

Plate 11, D, from an 8-day infection, shows another possible method of entrance. Here are two adjoining stomata in the lower epidermis, one (*f*) cut across the guard cells, the other (*d*) parallel to them. In both stomata are hyphae filling the stomatal aperture, and above, at *a*, *b*, *c*, and *e*, are binucleate or trinucleate fungus cells. The finding of sporophytic cells next to a stoma is not in itself proof that the nuclei entered at that stoma, for the sporophytic hyphae might have grown in from some other point. The likelihood is, however, that fusions frequently take place at stomata in the lower surface of the leaf.

Reference was made earlier (pl. 6, M) to the frequent occurrence of intracellular hyphae that grow through the epidermal cell to its outer wall and stop there, without forming subcuticular growth. The example shown in Plate 11, E, suggests that these also may serve to admit spermatial nuclei. The present instance occurred at a young spermogonium at the lower epidermis of a 7-day infection. A branch from a young paraphysis (*b*, *c*) grew out intracellularly through an epidermal cell and flattened itself against the outer epidermal wall at *d*. No opening can be seen in the host cell wall, but the hypha (*d*) contains six minute nuclei, and the paraphysis (*c*) of which it is a branch contains three full-grown nuclei. The inference is that there were spermatia on the lower leaf surface (later washed off during fixation or dehydration) and that the extremely small spermatial nuclei passed through the wall of the epidermal cell into the hypha. The trinucleate cell at *a* in the interior of the spermogonium remains unexplained.

It is with some hesitation that Plate 11, F, is added to the list of possible modes of entrance, for it differs markedly from the rest. It shows part of the brush of paraphyses of a spermogonium from a 12-day infection. At *h* is an epidermal cell of the leaf, and from *a* to *g* the extruded part of a group of paraphyses with the adherent exudate. Some of the spermatia (perhaps introduced spermatia) have expanded, rounding out from their original capsule shape to a sphere (*a*). One of these swollen spermatia at *b* has pushed out a fine germ tube, probably utilizing the sirup of the exudate as food, and the tube has grown down along the surface of a paraphysis to *e*, and on to *g*, where it passes behind other cells. At *c* is a similar germ tube, the upper end of which was removed in sectioning. It can be traced from *c* down to *f* and on to *i* (inside the host plant), beneath the epidermal cell. It contains a nucleus at the growing tip at *i*. At *d* there is a coarser structure,

EXPLANATORY LEGEND FOR PLATE 12

A.—Portion of subepidermal weft of 12-day infection: *a*, Spermatia on upper epidermis (*b*); *c*, *e*, *i*, *j*, and k, sporophytic cells in the mycelial weft; *d*, *f*, and *h*, remnants of earlier hyphae; *g*, trinucleate hypha between palisade cells. × 1,020.

B.—Twelve-day infection: *a*, Spermatia on epidermis (*b*) at outer margin of subepidermal space (*c*); *d*, *f*, and *g*, successive cells of hypha with 2, 3, and 2 nuclei, respectively; *e*, palisade. × 1,020.

C.—Thirteen-day infection: *a*, *b*, *c*, *e*, and *f*, Sporophytic mycelium in air spaces of mesophyll above aecium; *d*, binucleate cell in aecium. × 1,020.

D.—Sporophytic hypha, with 2-1-3 nuclei in successive cells, from 13-day infection. × 1,020.

E.—Hypha, from sporogenous area of aecium of 13-day infection, with 2-2-3 nuclei in successive cells. × 1,020.

F.—Mycelial cell of 13-day infection, with two large and two small nuclei: *a*, cell with large and small nucelei. × 1,020.

G.—Detail of 13-day infection: *b*, Hypha between epidermal cells and intracellular branch (*c*); *a*, continuation of same hypha above, with four nuclei in one cell. × 640.

H.—Thirteen-day infection: *a*, *b*, *c*, *d*, and *e*, Hypha found between palisade cells; the successive cells have 4, 0, and 3 nuclei, respectively. × 1,020.

I.—Fusion cell from sporogenous area of aecium of 13-day infection. × 1,020.

J.—Multinucleate cells from aecium containing fusion cell shown in I. × 1,020.

144776—32——2

probably an abnormal or decadent paraphysis. These germ tubes are extremely minute (one-third to one-half the diameter of a paraphysis), and only when they are sharply stained can they be seen at all. In safranine and in methylene blue the paraphyses tend to stain pink and the germ tubes blue. Of course it is possible that these are minute spores of some other fungus that alighted accidentally on spermogonial exudate and started to grow there, but no other evidence of secondary fungi has been seen. Moreover, the enlarged spermatia at *a* are evidence against that theory. If when introduced spermatia adhere to paraphyses (as must frequently happen) they can grow down through the ostiole and combine there with native hyphae, it would go far to explain the rather frequent occurrence (pl. 11, A, *i*; E, *a*) of binucleate or trinucleate cells in the body of the spermogonium and along its walls.

Plate 11, B, shows still another possibility. When two infections of opposite sex are located close together in the leaf and become confluent as they grow, open aecia are formed on both infections. In such cases the diploid growth may start by means of the two kinds of spermatia on the leaf surface, and probably does so. Plate 11, B, suggests that the sporophyte generation may also originate within the leaf by hyphal fusions. The two hyphae (pl. 11, B) are tangent; at the point of contact there is an open passageway from one to the other, and the nuclei of the two cells are in contact. The infection is a little too old (13 days) for a determination of its multisporidial origin by means of the primary hyphae, for the latter have so far degenerated that they can not be distinguished with certainty from haustoria and intracellular receptive hyphae, which also occur in epidermal cells. The grouping of the spermogonia, however, strongly suggests two centers of growth, and the probability is that two infections overlapped here.

A branched hypha (pl. 15, A, *a*), found beside a young aecium in another infection, is doubtful; but it, too, is at least open to interpretation as a hyphal fusion.

By whatever method the sporophyte starts, it soon spreads from the point or points of origin; and occasional hyphae with cells of more than one nucleus are found scattered among the haploid hyphae.

As previously stated, when spermogonia in the young infection form on the subepidermal mat of hyphae, they lift the epidermis well above the palisade, leaving an open space in the leaf in the intervals between spermogonia. The hyphae of this open space are often stretched and broken in this process and usually die. (Pl. 5, B and D.) This space, however, soon fills with secondary growth.

Since the sporophyte generation may start at various points along either the upper or lower epidermis, it naturally spreads from those points in the comparatively open spaces of the subepidermal weft of mycelium. Plate 12, A, shows a portion of this subepidermal growth.

EXPLANATORY LEGEND FOR PLATE 13

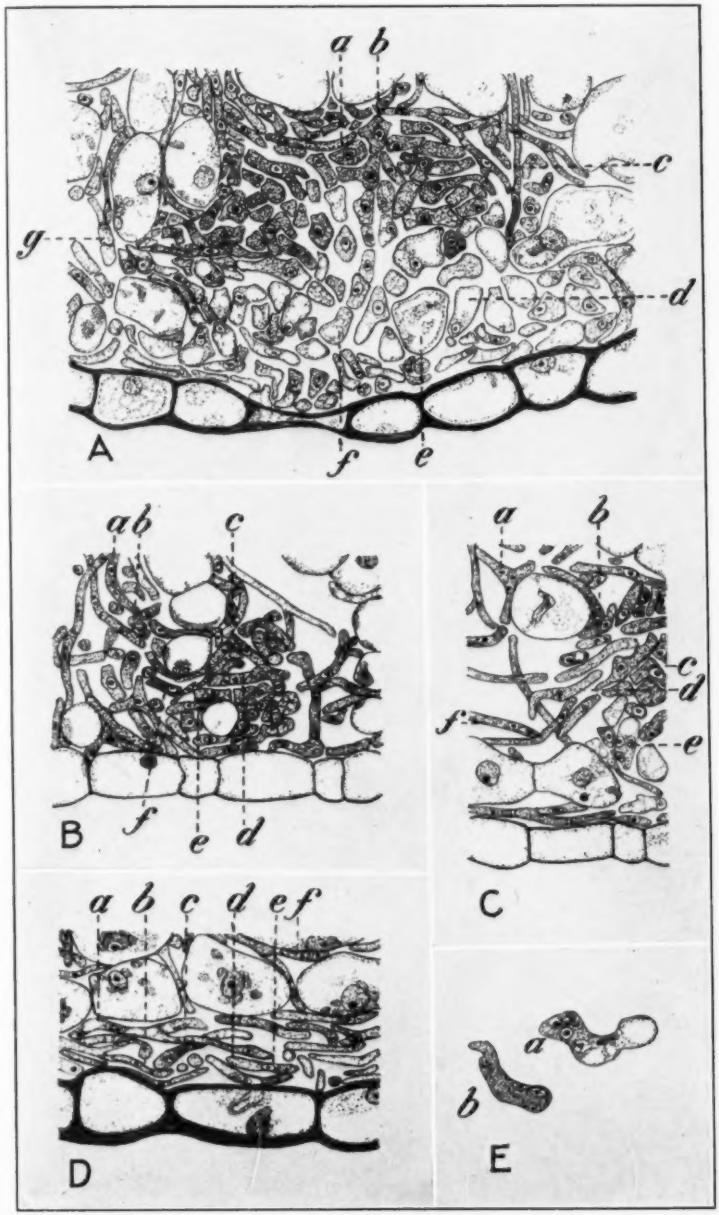
A.—Aecium of uninucleate cells: *a* and *c*, upper part, of denser cells; *d*, lower part, of large vacuolated cells; *b* and *f*, extremities of hypha extending through both areas; *e*, dead host cell; *g*, marks location of Plate 13, C. × 640.

B.—Beginning of fertile aecium in 8-day infection: *a*, *b*, *c*, *d*, *e*, and *f*, Sporophytic cells. × 640.

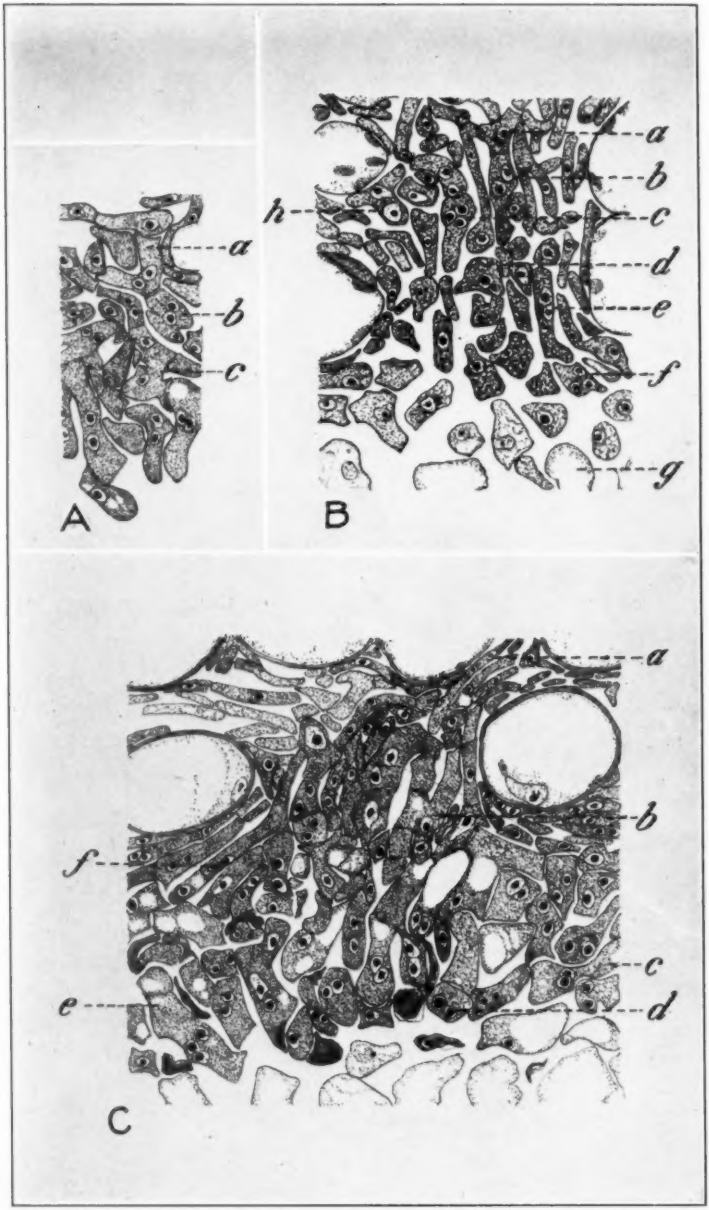
C.—Detail from section adjoining A, at *g*: *a*, *b*, *d*, *e*, and *f*, Sporophytic mycelial cells; *c*, edge of aecium. × 640.

D.—Portion of subepidermal weft above lower epidermis from 12-day infection: *a*, *b*, *c*, *d*, *e*, and *f*, Sporophytic cells. × 640.

E.—Two multinucleate mycelial cells (*a*, *b*) thickened like aecial cells. × 1,020.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

Above the epidermis (*b*) is a group of spermatia at *a*. Below, at *g*, is the palisade layer of the leaf. Between epidermis and palisade is a broad space filled with a fresh growth of vigorous young hyphae, at least a third of which are diploid. In the interstices of this mat (*d*, *f*, *h*) are dead remnants of earlier hyphae.

Fresh hyphae grow down from the subepidermal weft, passing between the palisade cells and out into the air spaces of the spongy mesophyll beneath. The majority of these hyphae are composed of uninucleate cells. At Plate 12, A, *g*, however, is a trinucleate cell issuing from the weft. In Plate 12, B, a detail drawn from the thin marginal growth of another subepidermal mat, is a sporophytic hypha growing down from *d*, just beneath the epidermis, to *g*, in an air space below the palisade. There are dried spermatia on the leaf surface at *a*, and a section or so farther on a hypha traversing the epidermal cell to the surface evidently afforded means of entrance. Farther down in the same section, beyond the limits of the drawing, there are other diploid hyphae in mycelium above a young aecium.

Plate 13, D, shows a portion of the weft above the lower epidermis of the leaf. Here, too, is a large percentage of cells with more than one nucleus. From here, too, the sporophyte generation spreads into the air spaces of the mesophyll.

The sporophyte spreads rapidly from the point or points of origin. This can take place by ordinary growth of the diploid hyphae with a division of both nuclei at the origin of each new cell. It is probable, also, although difficult to prove, that the sporophyte can spread by the diploidization process of nuclear divisions and migrations described by Buller (7). The following data may have a bearing on this question:

It has been frequently observed that the number of nuclei in vegetative mycelial cells varies. The majority of sporophytic cells are binucleate, but trinucleate cells also are of frequent occurrence. (Pl. 12, A, *g;* B, *f;* C, *c*, *e;* H, *d;* pl. 13, C, *a;* D, *c*, *f*.) Occasionally, there are more than three nuclei in a cell. (Pl. 12, C, *f;* F, *a;* G, *a;* H, *b;* pl. 13, B, *b;* D, *e*.) Rarely, one of these multinucleate mycelial cells becomes greatly thickened (pl. 13, E, *a*, *b*), taking on the appearance of a cell in the sporogenous area of the growing aecium.

In the examples cited, the nuclei are not located in any regular way within the cell. They may be together near the center of the cell, or one nucleus may be at the end. Plate 12, H, *b*, shows one pair of nuclei at one end of the cell, another pair at the other end. Moreover, the nuclei of one cell are often of different sizes, as though they had not divided at the same time. It is fairly common to find one large nucleus and two or more small ones in the same cell. Plate 13, B, *a*, shows one large nucleus and a pair of small nuclei. Plate 13, B, *b*, shows a large central nucleus and two pairs of smaller nuclei. Plate 13, D, *e*, shows one large and three small nuclei. Plate 12, F, shows two full-grown nuclei and a pair of small ones.

EXPLANATORY LEGEND FOR PLATE 14

A.—Detail of sporogenous area of 14-day fertile aecium. Probable fusions at *a*, *b*, and *c*.

B.—Strip through upper half of an aecium of a 12-day fertile infection: *a*, *b*, *c*, *d*, *e*, *f*, and *h*, Sporophytic cells; *f* to *g*, looser space-making tissue.

C.—Upper central part of 12-day fertile aecium: Haploid and diploid hyphae enter at *a*, grow down, and spread to right and left; *b*, probable cell fusion; *c* to *e*, layer of basal cells forming; *d*, spore mother cell; *f*, 3-cell hypha. (All × 1,020.)

Furthermore, successive cells of the same hypha may not have the same number of nuclei. These hyphae make their way through the irregular air passages of the mesophyll, and it is seldom possible in sectioned material to trace a given hypha for more than one or two cells. Plate 13, D, shows two hyphae (*a*, *d*) with two nuclei in one cell and one nucleus in the next. In Plate 12, C, the cell at *b* has two nuclei and the next cell of the same hypha, as shown at *c*, has three. In Plate 12, B, the sequence in the hypha, as shown at *d*, *f*, and *g*, is 2–3–2. In Plate 12, D, it is 2–1–3. The hypha in Plate 12, H, is drawn in two parts from *a* to *c* and *d* to *e*, and the sequence here is 4–0–3. The middle cell in this sequence has no nucleus. This is not rare.

Another point of interest in this connection is that the septa delimiting the cells in these hyphae are frequently vague and hard to see. The end of one cell and the beginning of the next is more often recognized by a definite break in the cytoplasm than by an easily visible cross wall. This condition would facilitate nuclear migrations.

All these irregularities are difficult to explain on the basis of the apical growth of a diploid hypha with a regular conjugate division at the origin of each new cell, but are readily explainable as the divisions and migrations of one or more introduced nuclei within a ready-formed haploid hypha. Moreover, a hypha may show an irregular combination of the two processes; it may continue to grow while nuclei are moving along inside it.

If sporophytic mycelium is present when an aecium starts, the sporophytic and gametophytic components are intimately mixed in the aecium from its very inception. Plate 13, B, shows a very young aecium containing a liberal admixture of cells (*a–f*) with two or more nuclei. On the other hand, an aecium may begin in the absence of the sporophytic component and be invaded by it later on in its development. Plate 13, A, shows a vigorous young aecium composed entirely of uninucleate cells. At one edge of this aecium in an adjoining section, however, at a position corresponding to Plate 13, A, *g*, there are several sporophytic hyphae. This area is drawn separately as Plate 13, C, showing the edge of the aecium at *c*, and cells with two or more nuclei at *a*, *b*, *d*, *e*, and *f*.

Plate 12, C, already referred to, shows a similar arrangement, including the upper edge of an aecium and the mycelium in the air spaces of the mesophyll tissue above it. The mycelium includes several sporophytic hyphae composed of cells with from one to five nuclei. No direct line of sporophytic cells can be traced from the mycelium into the aecium, but one hypha (*b–c*) has reached its edge.

The aecium (pl. 12, C), which is somewhat more advanced than the one in Plate 13, A, is uninucleate throughout, except for three or four cells in the sporogenous area. These, one of which (pl. 12, C, *d*) is included in the drawing, suggest strongly by their shape that they have just been formed by the fusion of two uninucleate cells. This aecium was part of the same infection from which the hyphal fusion in Plate 11, B, was drawn. As stated before, it is probable that two mycelia of opposite sex intermingle here. It may well be that haploid hyphae of both sorts entered into the formation of this aecium and are uniting here.

Further evidence of the invasion of an aecium after it is well started is shown in Plate 14, B and C. Plate 14, B, shows a narrow

median strip through the upper half of an aecium. At the lower end of the drawing (*f–g*) is the edge of the looser, space-making tissue, and above this area are the sporogenous cells. All through this upper region are cells with two or three nuclei (pl. 14, B, *a*, *b*, *c*, *d*, *e*, *f*), no one of which looks as though it were the immediate product of fusion. The shape and sequence of these cells suggest that sporophytic hyphae have grown in from some point of origin above the aecium.

Plate 14, C, shows the upper central part of a slightly older aecium of a 12-day infection, in which the evidence is even stronger of an invasion from above. Apparently a mixture of diploid and haploid hyphae entered at *a*, grew downward, branching freely, and then spread to right and left. With the exception of the cell at *b*, none of the cells appears to be the direct result of fusion.

In many cases, however, fusions do continue to occur in the lower part of the sporogenous area. In Plate 14, A, the cells at *a*, *b*, and *c*, indicate by their shape that two cells have combined to produce them, although here, as in some of the other cases cited, there are binucleate cells in the mycelium outside the aecium. Plate 15, A, *d* and *e*, shows other cells from the sporogenous area whose shape indicates fusion.

At this time binucleate cells in the aecium are rapidly increasing in numbers, trinucleate cells are less common, and cells with more than three nuclei are exceptional. Hyphae with different nuclear content in successive cells may still be found. In Plate 12, E, the sequence is 2–2–3; in Plate 14, C, *f*, it is 1–2–1; and in Plate 15, A, *b*, it is 4–2–3.

The haploid, unisexual aecium, if left to itself (pls. 8, 9, 10), develops to a certain stage, becomes differentiated into areas, forms irregular multinucleate cells in what corresponds to the sporogenous area, then deteriorates and dies. In the earlier stages of this development an invasion of sporophytic hyphae or perhaps even of haploid hyphae of opposite sex may become incorporated in its growth and change it to a normal fertile aecium.

Just how late in development such an invasion can become effective is not certain. One aecium had reached the earlier signs of deterioration. The cells of the sporogenous area were loosely spaced and had become less dense in content. The first irregular multinucleate cells (see pl. 12, J) had developed into typical forms. But several sporophytic hyphae were found extending into this aecium along its upper border, and near to these occurred the cell shown in Plate 12, I, which strongly suggests fusion between a slender invading hypha and a local cell. This aecium might be able to develop spores in normal fashion.

Spore formation begins about the twelfth day or, more rarely, as early as the tenth day. Plate 14, C, shows the layer of basal cells forming along the line from *c* to *e*. Thick heavy cells are beginning to grow down in more or less parallel alignment at the boundary of the space-making tissue. The first spore mother cell has been cut off at *d*.

A slightly more advanced stage (pl. 15, B) shows a part of the sporogenous area of a 12-day infection and the edge of the space-making tissue, (*d–e*). Sporophytic cells throughout the sporogenous area are growing downward toward the space-making tissue. Some of these (pl. 15, B, *b*, *f*) start at the boundary between the two areas and push out into the looser tissue below. Others higher up (*a*, *c*, *g*, *h*) grow downward and will eventually come into line with the former.

A majority of the sporophytic cells are now binucleate, but a few (pl. 15, B, *b;* D, *f;* E, *b, f;* C, *b*) have 3, 4, or even 5 nuclei.

This growth downward of a sporophytic cell may begin at one end or at any point along its length, probably varying according to the available space for such growth. Plate 15, E, *a* and *b*, and Plate 16, B, *d*, show the end of the cell pushing down. In Plate 15, B, *b*, the best opportunity for growth is near one end. Plate 15, B, *h*, shows the downward growth arising as a median branch, giving to the hypha a 3-prong shape. Plate 14, B, *h*, and Plate 15, D, *a–b*, show other triangular cells, which will give rise to the "2-legged" cells frequently figured in other rusts and shown in Plate 16, A, *a*.

Ordinarily, in fertile aecia making definite progress toward spore formation, the cells with more than one nucleus do not die. Plate 15, C, shows (in lower magnification than pl. 15, B) a detail from a sporogenous area, with several dead cells suggestive of those found in sterile infections, in a 14-day infection, the other aecia of which have formed young spores.

Plate 15, D, shows another detail from a sporogenous area. Some of the upper sporophytic cells, as at *c*, are still inactive; others (*d*) are beginning to grow down; and one (*e*) has formed the first spore mother cell. At *f* is a trinucleate cell with the scant cytoplasm characteristic of the space-making tissues.

Plate 15, E, already referred to, shows still other details. Some of the sporophytic cells (*i*) have not started to push down, others (*a, b*), have just started, while still others (*c, d*) have a fully formed basal cell, which has formed the first spore mother cell. In one of these, both the basal cell (*f*) and the first spore mother cell (*e*) are trinucleate.

When basal cells begin to carry on nuclear and cell divisions, their protoplasm concentrates in the lower end of the cell, leaving the upper end vacuolate. In a young sporogenous area all the cells have dense contents. By the time the sporophytic growth dominates the area the remaining gametophytic cells are clear and almost empty (pl. 16, B, *c, f*), contrasting sharply with the binucleate cells of the same region.

Mention has been made of what appear to be cell fusions in the sporogenous area of the aecium (pl. 12, C, *d;* I; pl. 14, A, *a, b, c;* C, *b;* pl. 15, A, *d, e*) before the onset of spore formation. Even in the later stages of development fusions can be found. In Plate 15, A, *c*, appearances indicate that two young basal cells have fused; the combination cell has six nuclei of different sizes. In Plate 16, B, *e*, two basal cells joined; apparently each contained at least two nuclei at the time of joining. In Plate 16, A, *b, c,* and *d*, three young spore mother cells of adjoining spore chains have fused; the combination cell has eight nuclei of different sizes. It is evident that

EXPLANATORY LEGEND FOR PLATE 15

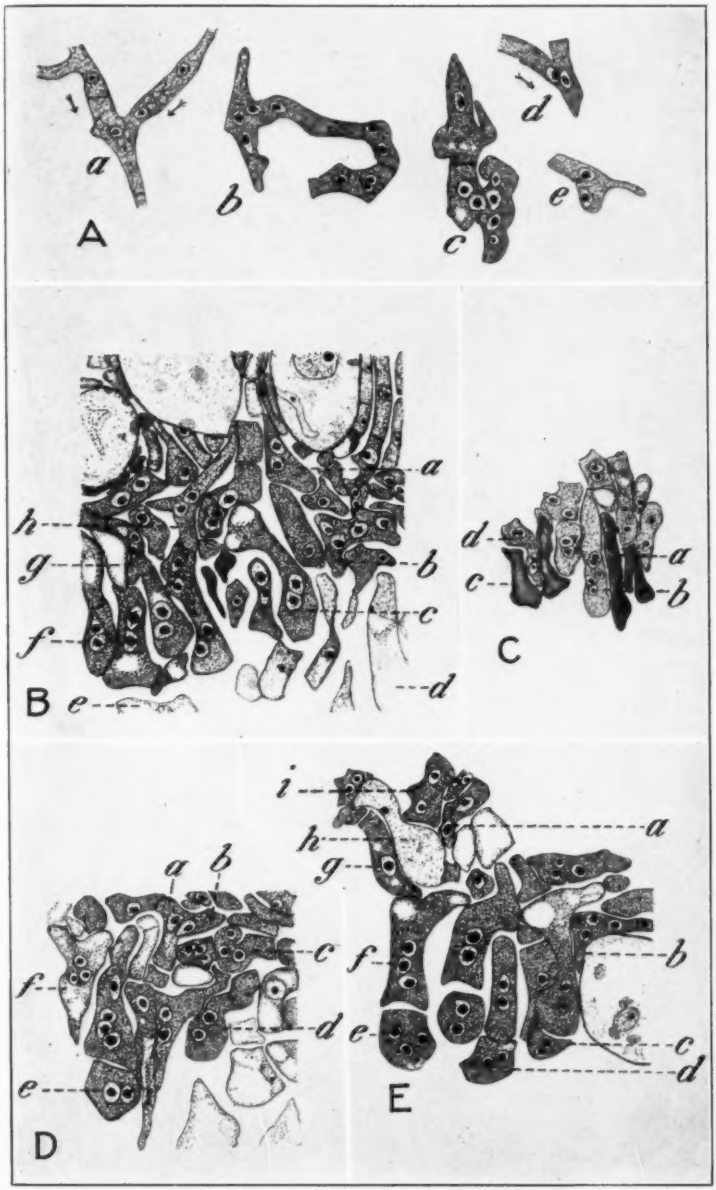
A.—Details from 12-day fertile infection: *a*, Possible hyphal fusion; *b*, hypha from sporogenous area, with quadrinucleate, binucleate, and trinucleate cells; *c*, fusion of basal cells; *d* and *e*, probable cell fusions in sporogenous area. Arrows indicate direction of growth. × 1,020.

B.—Portion of sporogenous area of a 12-day fertile infection: *a, b, c, f, g,* and *h*, Basal cells forming; *d* to *e*, border of space-making tissue. × 1,020.

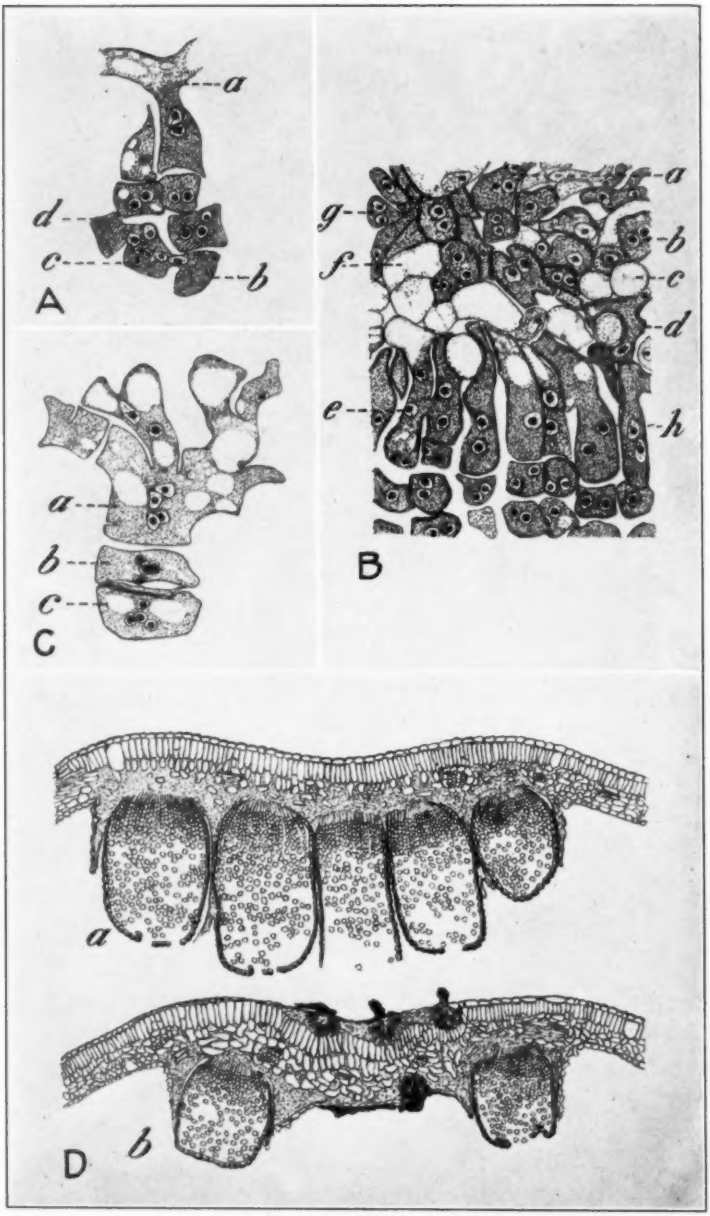
C.—Detail of sporogenous area of 14-day fertile infection: *a* and *c*, Dead basal cells; *b* and *d*, living basal cells. × 640.

D.—Detail of sporogenous area of 12-day fertile infection: *a, b, c, d,* and *f*, Basal cells forming; *e*, spore mother cell. × 1,020.

E.—As in D: Younger basal cells starting at *a* and *b;* fully formed basal cells and first spore mother cells below at *c, d, e,* and *f; g*, binucleate cell, one at *i* not yet started; *h*, dying haploid cell. × 1,020.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

the presence of two or more nuclei in a cell does not always prevent further fusions. Plate 16, C, shows evidence of this; a giant basal cell (*a*) has formed, evidently by multiple fusion, and is giving rise to a giant spore chain (*b*, *c*), of quadrinucleate cells. These irregularities are exceptional. The great majority of basal cells are binucleate, act as independent units, and produce chains of binucleate spores.

Under favorable conditions there are open aecia by the eighteenth day. If the infection has not been cramped by the crowding together of several infections nor deformed by encountering a large vein, it is circular in outline. The group of spermogonia or the denuded area formerly occupied by spermogonia is at the center of the circle on the upper surface. There is a similar, but smaller, spermogonial area at the lower surface. Opening on the lower surface are the aecia. Plate 16, D, shows two sections through a small 18-day infection. A central section (pl. 16, D, *b*) shows spermogonia (no longer functioning) at the center, flanked on either side by aecia. A section nearer the margin (pl. 16, D, *a*) shows a continuous row of aecia.

DISCUSSION

Although *Puccinia coronata*, *P. graminis*, and *P. triticina* (*1*, *2*) differ in many respects, the entry of the sporidium into the epidermal cell and the formation of the primary hypha and its mode of branching are very similar in the three species.

The first difference appears when branches from the primary hypha penetrate into the subepidermal region. In *Puccinia graminis* and *P. triticina* these hyphae grow downward at once into the spongy mesophyll and feed and spread there for several days; then a new growth of hyphae returns to the subepidermal region for the formation of spermogonia. In *P. coronata* the dominant growth for the first week is subepidermal. The hyphae force a passageway between epidermis and palisade and spread, forming a more or less circular stroma on which the spermogonia develop. From any point in this mat, hyphae may grow downward into the mesophyll.

This formation of a subepidermal weft of hyphae between epidermis and palisade depends upon the ability of *Puccinia coronata* to split apart the host cell walls and force a passage between them. The absence of a natural intercellular space is not an insurmountable obstacle, in thin-walled tissue at least, which, even in the smaller veins, is traversed by hyphae.

The aecia in a sterile infection of *Puccinia coronata* regularly form multinucleate cells, although the infection as a whole must be regarded as gametophytic. Bensaúde (*4*) and Sass (*24*), in studies of agarics, record the regular occurrence of a multinucleate phase in the young

EXPLANATORY LEGEND FOR PLATE 16

A.—Detail of spore chains from a 12-day fertile infection: *a*, 2-legged cell; *b*, *c*, and *d*, fusion of three spores. × 1,020.

B.—Detail from sporogenous area of 12-day fertile infection: *a*, *b*, and *g*, Dense sporophytic cells above; *c* and *f*, nearly empty gametophytic cells; *d*, new basal cell forming; *e*, fusion between basal cells; *h*, basal cells with young spore chains. × 1,020.

C.—Twelve-day fertile infection: *a*, Giant basal cell formed probably by multiple fusion, giving rise to a spore chain of giant quadrinucleate cells (*b*, *c*). × 1,020.

D.—Semidiagrammatic sections through an 18-day fertile infection: *a*, Lateral section showing a row of aecia; *b*, median section showing the patch of dead spermogonia at the center flanked by open living aecia. × 45.

primary or gametophytic mycelium. Lindfors (*18*) found that in *Trachyspora alchimilliae* (Pers.) Fckl. the uninucleate mycelium of the primary uredo generation may become multinucleate in the small intercellular spaces of the embryonic tissues of the host, but becames uninucleate again as those tissues expand. All these instances of multinucleate cells in the haploid generation occur in vegetative mycelium; they are therefore not directly comparable to the present case.

It is not known whether multinucleate cells similar to those of *Puccinia coronata* are to be found in unisexual aecia of other species of rusts. Until the announcement by Craigie (*11*), in 1927, of heterothallism in the rusts, it was not even known that unisexual infections existed. There are many references in the earlier literature of the rusts to the presence of multinucleate cells in aecia, and they are sometimes spoken of as "pathological." Olive (*23*) finds "a multinucleated stage" in a large proportion of 50 species examined. It would be interesting to know whether some of these occurred in sterile aecia not recognized as such.

In *Puccinia coronata* the multinucleate cells in the sterile aecium form at the time and in the place where spore-forming activities would be going on in a fertile aecium; their formation seems to be a futile expression of a reproductive tendency. The sporogenous area in a fertile aecium is richly supplied with food. A sterile aecium is similarly organized, and possibly the nutriment supplied to this area parallels, for a time at least, the supply that would have been needed for spore formation. Apparently, cells in this area in the unisexual aecium can grow and carry out the rapid nuclear divisions that would accompany spore formation, but can not form the spores. The resulting multinucleate cells become badly overgrown and irregular in shape and sooner or later deteriorate and die.

There is some evidence in the literature on rusts that development does not always stop at this point in the unisexual aecium. Moreau (*19, 20*) reported the widespread occurrence of a uninucleate variety of *Endophyllum euphorbiae* (DC.) Wint. Mycelium, aecia, and aeciospores were haploid throughout. Haploid aecia with regular spore chains of uninucleate aeciospores have been found by Kursanov (*17*) in three different collections of *Aecidium punctatum* Pers. The aecia fully resembled sporophytic aecia in all morphological details. The later history of these uninucleate aeciospores is not known. Kursanov (*17*) also found uninucleate aeciospores in *A. leucospermum* DC. These spores were capable of germination, but no infections were obtained. Dodge (*14*) found in the short-cycle rust *Caeoma nitens* Schw. a strain without spermogonia or with only vestigial spermogonia. There were no cell fusions at the base of the aecia; the spores were uninucleate and germinated by producing 2-cell promycelia with two sporidia.

The foregoing data are open to several interpretations. One possibility is that, when fertilization fails to take place, the unisexual aecium stops at different points in its development in different rusts. In *Puccinia graminis* and *P. triticina* the haploid aecium forms and the sporogenous area takes shape, but with rare exceptions the cells of the latter remain uninucleate and soon become impoverished and die. In *P. coronata* the sporogenous area of the unisexual aecium lives longer and its cells may carry out several nuclear divisions, but ultimately it dies without forming spores. In *Endophyllum euphor-*

biae, *Aecidium punctatum*, *A. leucospermum*, and *Caeoma nitens*, the haploid aecium can develop to the spore-forming stage and produce haploid spores.

Andrus (*3*) believes the hyphae that emerge upon the surface of the leaf are trichogynes, growing directly from "eggs" in the sporogenous layer of young aecia. He says (*3*, *p. 567*):

> At an early stage previous to fertilization, when the aecial primordia are in process of formation, that is, 8 to 12 days after inoculation, no hyphae are found in stomata or protruding between epidermal cells.

In *Puccinia coronata*, on the contrary, a mycelium so young that it has not yet reached the lower epidermis of the leaf and sometimes has not even started to form spermogonia may nevertheless have formed several emergent hyphae (pl. 6, B and C) growing up between epidermal cells and along under the cuticle. These can not have grown from aecia, since aecial primordia do not start to form until two or three days later.

In *Puccinia coronata*, hyphae may emerge upon the surface of the leaf at any point in the infected area after the fourth or fifth day of development. They form at the upper surface first and at the lower surface later, when the parasite becomes established there. A receptive hypha may grow out from vegetative mycelium, or it may start as a branch from a young paraphysis in a spermogonium or from any point in the subepidermal weft of hyphae, or it may grow from a young aecium to a stoma adjoining it. Relatively few of these receptive hyphae can be traced directly to an aecium. The emergent hyphae function as trichogynes in receiving the spermatial nuclei, but they show no further similarity to the trichogynes described in other fungi and in algae.

It should be pointed out, however, that in a monosporidial infection all the hyphae belong to one connected system. In that sense an emergent hypha, no matter how remote it may be from an aecium, is still connected with it, after aecia begin to form, by a series of hyphae. If the process of "progressive diploidization" described by Buller (*7*) should be proved to exist in rusts, it would explain how nuclei received at any point in the mycelium could be transmitted by successive nuclear divisions and migrations to any other point. The route to an aecium might be fairly direct or might involve irregular detours, but it would be feasible, for the distances involved are small.

In *Puccinia graminis* (*1*), *P. triticina* (*2*), and *P. coronata*, it has been repeatedly observed that when the sporophyte generation is spreading from its point or points of origin mycelial cells may contain from one to five nuclei, an occasional cell may have no nucleus, the nuclei may be of different sizes and show different arrangements within the cell, septa are often ill-defined, and successive cells in a hypha often do not have the same number of nuclei. In the systemic infections of *P. suaveolens* Rostr., in the neighborbood of the spermogonia and the primary uredo, Kursanov (*17*) found a mixture of gametophytic and sporophytic hyphae. In the latter the number of nuclei in successive cells of the same hypha varied. He reports the following sequences: 3–1–2; 2–2–2–3–2–2–2–1; 2–3–3–3–3–2–2; 2–2–1; and 3–2–1.

This seeming chaos is difficult to account for on the basis of the regular apical growth of diploid hyphae, but if the surplus nuclei in

the cells of the sporophytic hyphae are transient occupants, on their way in the process of diploidizing ready-formed haploid mycelium, the apparent disorderliness takes on new meaning. On this basis, too, an explanation suggests itself for the seemingly casual cell fusions found at different stages in the development of the aecium, in some cases between cells already containing more than one nucleus. A sporogenous area, established in part by a rather plastic and variable system of nuclear migrations, would be apt to contain some unpaired nuclei. It is conceivable (but, of course, not proved) that in these last-minute cell fusions an interchange of nuclei takes place by which such nuclear imbalances are corrected.

Evidence is increasing that the sporophyte generation originates outside the aecium in some species, but how widespread in the Uredineae this will be found to be is a matter for conjecture only. The occasional 2-legged basal cell, so long regarded as positive proof of the origin of the sporophyte in the aecium, is open, in some cases at least, to other interpretations. Moreover, it is evident from studies of *Puccinia coronata* that the presence of an occasional supplementary cell fusion in the aecium prior to the formation of the basal cells does not exclude the possibility of earlier sporophytic mycelium outside the aecium. Detailed investigation of a large series of rusts is necessary before any generalizations can be attempted on this subject.

Bisexual mycelium may originate at the leaf surface where emergent hyphae meet spermatia, but the evidence is still scant that it can originate also within the leaf by hyphal fusions where two mycelia interlace. Even if hyphal fusions were common, they would be difficult to find; but it is probable that they do take place.

The sporophyte generation is known to originate by means of hyphal fusions in other groups of fungi. Bensaúde (*4*) found that in Coprinus the dicaryophyte begins with the fusion of a (+) and a (−) mycelial cell or of a mycelial cell and an oidium. This has since been found to be general in heterothallic hymenomycetes. A few cases of hyphal fusion are known in the ascomycetes. Gwynne-Vaughan and Williamson (*15*) found that in *Humaria granulata* Quel. all single-spore mycelia produce well-formed multinucleate oogonia, which, however, remain sterile and ultimately degenerate. No antheridia have been found. Although all the individuals are morphologically female, they may nevertheless be divided into 2 clean-cut groups, 1 (+) and 1 (−). When a (+) mycelium meets a (−) mycelium, hyphal fusions take place initiating bisexual mycelium, on which are formed fertile oogonia that produce ascogenous hyphae and asci.

In other ascomycetes fertilization in the oogonium has been questioned. Even the famous *Pyronema confluens* (Pers.) Tul. is questioned, for Moreau and Moreau (*21*) maintain that the nuclei of the antheridium and the trichogyne degenerate and that oogonial nuclei issue two by two to form the ascogenous hyphae. They state (*21, p. 91*) that "Ni chez les Champignons des Lichens, ni chez le Pyronema, ni chez le Sphaerotheca, ni chez le Polystigma, nous n'avons observé de fécondation à l'origine du périthèce," and believe in (*21, p. 93*) "l'absence de toute fécondation actuelle à la base des périthèces des Ascomycètes."

They admit the possibility in heterothallic forms, however, of hyphal fusions between (+) and (−) mycelia, by which nuclei of

the two sexes could be brought together. One may not accept these generalizations, but it is evident that there is increasing reason for belief that the origin of the sporophyte generation by hyphal fusions is fairly common in the higher fungi.

In *Puccinia coronata* the basal cells just ready to start spore chains are predominantly binucleate but may contain as many as four or five nuclei. This multinucleate condition was more common and more pronounced in *P. graminis* and *P. triticina*, in which young basal cells often contained 5 or 6 nuclei and occasionally 8 or 10. Similar precocious nuclear divisions were found by Colley (*10*) in basal cells of *Cronartium ribicola* Fisch. In the fertile aecium these prematurely formed nuclei are utilized later in spore formation. In the sterile aecium, in *P. coronata*, the nuclei merely accumulate in monstrous cells that then die.

SUMMARY

The sporidium of *Puccinia coronata* on a leaf of *Rhamnus cathartica* pushes out a short beak, which becomes attached to the epidermal cell, pierces the wall, and grows into the cell. The sporidial nucleus divides before entry. A primary hypha of four to six cells is formed in the epidermal cell. From each cell, in turn, a branch grows down into the subepidermal region.

For the first week the dominant growth of the rust is subepidermal, the hyphae separating the epidermis from the palisade and forming a weft of hyphae between the two layers. This weft spreads radially beneath the epidermis. From it hyphae grow down into the mesophyll. Later, a smaller stroma may form next the lower epidermis.

Each spermogonium begins as a small circular patch on the subepidermal stroma from which hyphae grow upward. Heavy upright cells at the center of this group serve as buffer cells, pushing up the epidermis. From the margin of the patch a circle of slender paraphyses curve upward and inward, the tips meet, then grow out together and pierce the epidermis forming the ostiole. Numerous slender spermatiophores grow in from the sides and base of the spermogonium and set free spermatia into the central cavity. Spermogonial exudate is not abundant, and spermogonia are not long-lived. At the same time hyphae near either leaf surface grow out to the surface, pushing into stomatal apertures, or between epidermal cells or even through an epidermal cell. These hyphae, which may come from vegetative mycelium, from a spermogonium, or from a young aecium, serve to receive spermatial nuclei.

Puccinia coronata is heterothallic. The unisexual infection, if carefully isolated, produces spermatia but no aeciospores. Aecia form but remain sterile.

The sterile aecium begins as a little nest of uninucleate cells near the lower epidermis. It grows and becomes differentiated into an upper half of dense cells and a lower half of looser cells. In the upper half are cells containing more than one nucleus. These cells grow and their nuclei divide, sometimes several times, but sooner or later they deteriorate and die.

When spermatia of opposite sex are transferred to the surface of an infected area, their nuclei enter the exposed tips of the receptive hyphae, initiating there the sporophytic generation. From these

points the sporophyte spreads by growth of diploid hyphae, probably also by nuclear divisions and migrations through ready-formed haploid hyphae.

The aecium that is to form spores may contain both sporophytic and gametophytic hyphae from the start, or may begin as a haploid, unisexual aecium and be invaded later by the sporophytic component. An occasional cell fusion is found in the fertile aecium, sometimes between uninucleate cells, sometimes between cells one or both of which contain more than one nucleus. Basal cells are predominantly binucleate, occasionally multinucleate. With but few exceptions the spores are binucleate.

LITERATURE CITED

(1) Allen, R. F.
1930. A cytological study of heterothallism in Puccinia graminis. Jour. Agr. Research 40:585–614, illus.

(2) ———
1931. A cytological study of heterothallism in Puccinia triticina. Jour. Agr. Research 44:733–754, illus.

(3) Andrus, C. F.
1931. The mechanism of sex in Uromyces appendiculatus and U. vignae. Jour. Agr. Research 42:559–587, illus.

(4) Bensaúde, M.
1918. Recherches sur le cycle évolutif et la sexualité chez les basidiomycètes. 156 p., illus. Nemours.

(5) Blackman, V. H.
1904. On the fertilization, alternation of generations, and general cytology of the Uredineae. Ann. Bot. [London] 18:[323]–373, illus.

(6) ——— and Fraser, H. C. I.
1906. Further studies on the sexuality of the Uredineae. Ann. Bot. [London] 20:[35]–48, illus.

(7) Buller, A. H. R.
1930. The biological significance of conjugate nuclei in Coprinus lagopus and other hymenomycetes. Nature [London] 126:686–689, illus.

(8) Christman, A. H.
1905. Sexual reproduction in the rusts. Bot. Gaz. 39:267–275, illus.

(9) ———
1907. The nature and development of the primary uredospore. Wis. Acad. Sci., Arts, and Letters, Trans. 15:[517]–526, illus.

(10) Colley, R. H.
1918. Parasitism, morphology, and cytology of Cronartium ribicola. Jour. Agr. Research 15:619–660, illus.

(11) Craigie, J. H.
1927. Experiments on sex in rust fungi. Nature [London] 120: 116–117, illus.

(12) ———
1927. Discovery of the function of the pycnia of the rust fungi. Nature [London] 120:765–767.

(13) ———
1928. On the occurrence of pycnia and aecia in certain rust fungi. Phytopathology 18:1005–1015, illus.

(14) Dodge, B. O.
1924. Uninucleated aecidiospores in Caeoma nitens and associated phenomena. Jour. Agr. Research 28:1045–1058, illus.

(15) Gwynne-Vaughan, H. C. I., and Williamson, H. S.
1930. Contributions to the study of Humaria granulata Quel. Ann. Bot. [London] 44:127–145, illus.

(16) Hanna, W. F.
1929. Nuclear association in the æcium of Puccinia graminis. Nature [London] 124:267.

(17) KURSANOV, L.
1922. RECHERCHES MORPHOLOGIQUES ET CYTOLOGIQUES SUR LES URÉDINÉES. Bul. Soc. Nat. Moscou (n. s.) 31:1–129, illus.
(18) LINDFORS, T.
1924. STUDIEN ÜBER DEN ENTWICKLUNGSVERLAUF BEI EINIGEN ROSTPILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTSPUNKTEN. Svensk Bot. Tidskr. 18:1–84, illus.
(19) MOREAU, Mme. F.
1911. SUR L'EXISTENCE D'UNE FORME ÉCIDIENNE UNINUCLÉÉE. Bul. Soc. Mycol. France 27:[489]–493, illus.
(20) ———
1915. NOTE SUR LA VARIÉTÉ UNINUCLÉÉE DE L'ENDOPHYLLUM EUPHORBIÆ (DC.) WINTER. Bul. Soc. Mycol. France 31:[68]–70, illus.
(21) MOREAU, F., and MOREAU, Mme. F.
1930. LE DÉVELOPPEMENT DU PÉRITHÈCE CHEZ QUELQUES ASCOMYCÈTES. Rev. Gén. Bot. 42:65–98, illus.
(22) NEWTON, M., JOHNSON, T., and BROWN, A. M.
1930. A PRELIMINARY STUDY ON THE HYBRIDIZATION OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Sci. Agr. 10:721–731, illus.
(23) OLIVE, E. W.
1910. THE PRESENT STATUS OF THE CYTOLOGY OF THE RUSTS. Science (n. s.) 31:437–438.
(24) SASS, J. E.
1929. THE CYTOLOGICAL BASIS FOR HOMOTHALLISM AND HETEROTHALLISM IN THE AGARICACEAE. Amer. Jour. Bot. 16:663–701, illus.
(25) STAKMAN, E. C., LEVINE, M. N., and COTTER, R. U.
1930. ORIGIN OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS THROUGH HYBRIDIZATION AND MUTATION. Sci. Agr. 10:707–720.
(26) WATERHOUSE, W. L.
1929. A PRELIMINARY ACCOUNT OF THE ORIGIN OF TWO NEW AUSTRALIAN PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Linn. Soc. N. S. Wales, Proc. 54:96–106, illus.

SOME PHYSIOLOGICAL STUDIES OF POTATOES IN STORAGE[1]

By R. C. WRIGHT

Physiologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The purpose of the investigation begun in 1924 and described herein was to determine the changes in sugar content, the rate of respiration, and the intercellular or internal atmospheric composition of potatoes stored at different temperatures and sampled at succeeding periods during storage. Studies along these lines were carried on simultaneously, or as nearly so as practicable. Investigations were made also on changes in potatoes that had been held in storage at 32° F. for a given time and had then been transferred and held at several different higher temperatures, and in potatoes that had been frozen.

MATERIAL USED

The potatoes used in these investigations were Irish Cobbler, Green Mountain, and Triumph varieties true to name and grown by the United States Department of Agriculture at Presque Isle, Me. They were well matured, carefully selected for uniformity of size, and were free from evidence of disease. In 1924 the varieties were harvested on September 24 and stored in crates in a potato storage house at a temperature of about 50° F. until shipped. They were shipped in barrels by freight directly to Washington, D. C., and arrived at the laboratory on November 1.

On arrival at Washington all the potatoes of each variety were carefully emptied on the floor in such a way as to mix together the contents of the barrels. Potatoes were then selected from different locations in the pile in order to secure composite samples as nearly comparable as possible. In handling the potatoes care was taken to avoid skinning or other mechanical injury that might cause physiological disturbance. As the potatoes were well matured and the skins rather firm, practically no skinning occurred.

CHANGES IN POTATOES DURING CONTINUOUS STORAGE AT DIFFERENT CONSTANT TEMPERATURES

On November 5 lots of each of the three varieties were put in storage at controlled temperatures of 32°, 36°, 40°, 50°, 60°, and 70° F. and allowed to remain continuously for periodic sampling. The relative humidity was maintained at approximately 85 per cent in order to prevent undue loss of moisture.

[1] Received for publication Feb. 18, 1932; issued November, 1932.

SUGAR CONTENT

In order to determine the initial sugar content for each variety, representative composite samples were taken for analysis at the beginning of the experiment. Following this, samples were taken periodically, on November 26, December 17, February 5, March 24, and June 1.

Cylindrical plugs about 15 mm in diameter were taken from each of about 12 representative potatoes by cutting with a cork borer from side to side through the center. The plugs from each lot were put through a sampling press that reduced the material to a fine pulp, which was then mixed by thorough stirring. Duplicate 50 g portions of each sample were weighed out and covered with approximately 85 per cent alcohol in 400 c c beakers and boiled for a few moments. The samples were then washed into 500 c c volumetric flasks, brought to volume, and allowed to stand, with occasional shaking, until all the samples had been taken.

After filtering, a 50 c c aliquot part of the alcoholic extract was evaporated in order to remove the alcohol. It was then brought to nearly 250 c c with water; 5 c c of neutral lead acetate was added for clearing; and the mixture was then brought to exact volume, filtered, and deleaded with sodium oxalate. Reducing sugar was determined in a 50 c c aliquot portion by using Bertrand's modification of the Munson and Walker method. To another 50 c c aliquot portion of the clarified and deleaded solution 5 c c of hydrochloric acid was added, and the whole brought to 100 c c. After inversion in the cold for 24 hours and then neutralizing, total sugar was determined on a 50 c c aliquot by the method previously used to determine the reducing sugar. Sucrose was then determined by difference. The averages, calculated on the basis of the weight of the fresh tissue and expressed as percentage of dextrose, are shown in Table 1 and in Figure 1.

TABLE 1.—*Progressive changes in sugar content of potato varieties stored at various temperatures*

[Sugars expressed as percentage of dextrose based on fresh weight]

IRISH COBBLER

Sampling date	Sugar percentage at storage temperature of—																	
	32° F.			36° F.			40° F.			50° F.			60° F.			70° F.		
	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars
Nov. 5 [a]	0.91	0.27	1.18	0.91	0.27	1.18	0.91	0.27	1.18	0.91	0.27	1.18	0.91	0.27	1.18	0.91	0.27	1.18
Nov. 26	2.21	2.03	4.24	2.01	1.43	3.44	1.65	.33	1.98	1.00	.24	1.24	.67	.27	.94	.82	.25	1.07
Dec. 17	3.47	1.13	4.60	2.21	1.19	3.40	1.76	.30	2.06	.79	.34	1.13	.72	.22	.94	.60	.23	.83
Feb. 5	4.57	1.29	5.86	2.75	1.00	3.75	1.35	.26	1.61	.78	.22	1.00	.50	.16	.66	.35	.22	.57
Mar. 24	4.42	1.45	5.87	2.88	1.36	4.24	1.61	.30	1.91	1.50	.15	1.65	[b]	[b]	[b]	[b]	[b]	[b]
June 1	3.87	1.90	5.77				[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
GREEN MOUNTAIN																		
Nov. 5 [a]	1.99	0.27	2.26	1.99	0.27	2.26	1.99	0.27	2.25	1.99	0.27	2.26	1.99	0.27	2.26	1.99	0.27	2.26
Nov. 26	3.25	1.17	4.42	2.66	.78	3.44	1.21	1.24	2.45	1.54	.20	1.74	1.41	.20	1.61	1.46	.23	1.69
Dec. 17	4.68	1.91	6.59	3.57	1.16	4.73	1.37	.37	1.74				1.32	.33	1.65	1.17	.22	1.39
Feb. 5	5.23	1.81	7.04	3.43	2.16	5.59	1.96	.30	2.26	1.54	.17	1.71	.74	.22	.96	.77	.15	.92
Mar. 24	4.87	2.41	7.28	3.43	1.82	5.25	2.01	.51	2.52	.89	.29	1.18	[b]	[b]	[b]	[b]	[b]	[b]
June 1	4.28	2.40	6.68				[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
TRIUMPH																		
Nov. 5 [a]	1.35	0.30	1.65	1.35	0.30	1.65	1.35	0.30	1.65	1.35	0.30	1.65	1.35	0.30	1.65	1.35	0.30	1.65
Nov. 26	2.70	1.81	4.51	2.59	3.45	6.04	1.60	.25	1.85	1.04	.16	1.20	1.02	.18	1.20	1.15	.18	1.33
Dec. 17	4.12	2.42	6.54	3.20	2.03	5.23	.92	.28	1.20	1.28	.28	1.56	.93	.33	1.26	.87	.15	1.02
Feb. 5	4.07	2.79	6.86	3.34	2.34	5.68	1.99	.31	2.30	1.02	.20	1.22	.81	.15	.96	.65	.18	.83
Mar. 24	4.75	3.22	7.97	2.70	3.16	5.86	2.12	.49	2.61	.95	.23	1.18	[b]	[b]	[b]	[b]	[b]	[b]
June 1	4.35	2.01	6.36				2.21	.51	2.72	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]

[a] Before storage.

[b] Sprouting.

144776—32—3

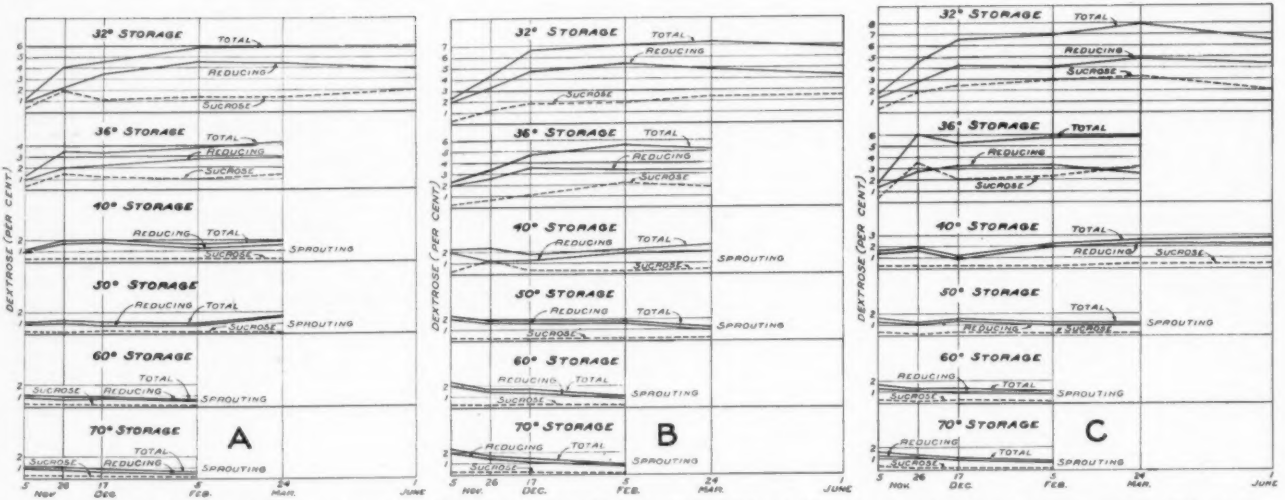


FIGURE 1.—Progressive changes in the sugar content of Irish Cobbler (A), Green Mountain (B), and Triumph (C) potatoes at various storage temperatures, degrees Fahrenheit

In all three varieties stored at 32° and 36° F., disregarding certain fluctuations, a general progressive increase in reducing sugar, sucrose, and total sugar was found at each sampling, including samples taken on March 24. This was followed by a tendency to decrease, as shown on June 1. The greatest amount of total sugar accumulated was at 32°. The sugar content of the Irish Cobbler increased from 1.18 to 5.87 per cent, Green Mountain from 2.26 to 7.28 per cent, and Triumph from 1.65 to 7.97 per cent, or percentage increases of 397, 222, and 383, respectively. (Table 1.) The most rapid increase in sugar occurred between the beginning of the experiment and the first sampling date, that is, during the first 21 days of storage. In potatoes stored at 40° there was apparently a slight general increase in sugar, but the quantity seemed to fluctuate from one sampling date to another. This fluctuation may have been due to analytical error or variation in sampling, although great care was taken at each sampling to select representative tubers from different locations in the baskets. With the exception of the Irish Cobbler variety, potatoes stored at 50° showed a general decrease in sugar on March 24. In this variety more sugar was found than at the beginning, but this is considered to be due to some experimental error. At 60° and 70° the total-sugar content in general decreased during storage to one-half or less. With certain exceptions, the tendency was for a loss of sugar, increasing in proportion as the temperature increased from 50° to 70°.

The relatively high sugar content of potatoes stored at 32° and at 36° F. explains much of the unpalatability due to excessive sweetness commonly recognized in tubers stored at low temperatures. Sweetness was readily detected by taste in uncooked specimens stored at these low temperatures, as compared with those stored at 40° or above.

RESPIRATION

In the same storage lots of Irish Cobbler and Green Mountain potatoes on which sugar analyses were made periodically, the relative rates of respiration, expressed as milligrams of carbon dioxide given off per kilogram of material per hour, were determined on three dates during the storage period. The method and apparatus used were similar to those described by Magness and Diehl (*8*).[2] The respiration tests were carried on in duplicate on approximately 3-kg samples. The first test was begun on November 7, two days after the potatoes were placed in storage; similar tests were begun on January 17 and on March 24. The duration of each test was between 300 and 400 hours. After each test the tubers used were discarded, and new groups of tubers were selected for each succeeding test. The results obtained at the storage temperatures indicated are presented in Table 2. The respiration rates, as determined in continuous runs of approximately 24 hours each throughout these tests, proved to be fairly uniform; for the sake of brevity only averages for entire test periods are given herein.

[2] Reference is made by number (italic) to Literature Cited, p. 555.

TABLE 2.—*Seasonal respiration of Irish Cobbler and Green Mountain potatoes in storage at various temperatures*

IRISH COBBLER

Test begun	Storage temperature	CO_2 per kilogram per hour	Duration of test	Weight of sample	
				Beginning	End
	°F.	*Mg*	*Hours*	*Kg*	*Kg*
Nov. 7	32	6.22	408	3.610	3.592
	36	9.28	380	3.522	3.510
	40	8.48	404	3.800	3.792
	50	5.92	387	2.675	2.661
	60	8.75	263	3.682	3.667
	70	8.91	405	3.575	3.549
Jan. 17	32	4.25	332	2.607	2.603
	36	4.66	288	2.651	2.636
	40	3.92	339	2.423	2.423
	50	4.35	338	2.514	2.514
	60	10.40	389	2.828	2.818
	70	12.42	285	2.993	2.952
Mar. 24	32	3.27	402	3.054	3.050
	36	4.52	405	3.274	3.272
	40	3.68	386	2.975	2.972
	50	4.50	388	2.415	2.411
	60	(a)	(a)	(a)	(a)
	70	(a)	(a)	(a)	(a)

GREEN MOUNTAIN

Nov. 7	32	5.85	408	3.512	3.502
	36	7.07	380	3.422	3.415
	40	5.11	404	3.660	3.651
	50	6.40	387	2.641	2.631
	60	8.63	386	3.650	3.632
	70	10.06	405	3.645	3.621
Jan. 17	32	4.20	332	2.508	2.508
	36	3.94	288	2.843	2.831
	40	3.57	339	2.752	2.752
	50	3.73	338	2.177	2.177
	60	5.80	289	2.855	2.845
	70	9.38	285	2.885	2.856
Mar. 24	32	2.99	402	2.997	2.994
	36	3.65	309	3.425	3.416
	40	2.93	386	3.422	3.420
	50	4.86	388	2.037	2.030
	60	(a)	(a)	(a)	(a)
	70	(a)	(a)	(a)	(a)

[a] Sprouting.

These results and those of Bennett and Bartholomew (*2*) and of Hopkins (*5*) show, as suggested by Gore (*3*), that the general rule of Van't Hoff (that the respiration rate doubles or trebles for each 10° C. (18° F.) rise in temperature) does not hold for low temperatures. In general, a greater rate of respiration was found at 36° F. than at 32° or 40° or even at 50° early in the storage season, little relation to sugar concentration being shown at these temperatures. Hopkins (*5*), working with the Russet Rural variety in storage at various temperatures, reported a more rapid rate of respiration at 32° than at any higher temperature up to 47.6°. In general, the rate of respiration at all temperatures decreased as the storage season advanced, except in the case of the Irish Cobbler variety stored at 60° and 70°, which showed an increase on January 17. Sprouting in this variety, noted soon after this test, may account for the increased respiration. Sprouting was noted in the Green Mountain variety at 60°; but it had apparently just begun, since at this time the growth was not so far advanced as in the Irish Cobbler variety.

COMPOSITION OF INTERNAL ATMOSPHERE

After the three varieties of potatoes had been in storage from November 5 to February 18, the constituents of the atmosphere in the intercellular spaces were determined. Unfortunately, no determinations were made at the beginning of the storage period in order to ascertain what changes took place during storage. However, the results presented in Table 3, representing the average of six tubers, show the composition of the internal atmosphere of each of the three varieties after 105 days of continuous storage at 32°, 36°, 40°, 50°, and 60° F.

TABLE 3.—*Percentage composition of the internal atmosphere of Irish Cobbler, Green Mountain, and Triumph potatoes after storage at various temperatures from November 5 to February 18*

Storage temperature (°F.)	Irish Cobbler			Green Mountain			Triumph		
	CO_2	O_2	N	CO_2	O_2	N	CO_2	O_2	N
32	12.0	18.4	69.6	9.0	20.5	70.5	17.2	13.9	68.9
36	17.8	15.9	66.3	17.3	16.3	66.4	14.7	18.1	67.2
40	12.7	18.0	69.3	10.3	18.4	71.3	9.6	26.3	64.1
50	7.2	18.4	74.4				9.9	13.8	76.3
60	10.4	17.5	72.1	8.3	17.1	74.6	15.3	18.4	66.3

The internal atmosphere was extracted by the method described and illustrated by Magness (*7*). For each potato variety, composite samples of gas were obtained from six representative potatoes taken from each storage. A cylindrical plug about 15 mm in diameter was taken from each potato by cutting with a cork borer from side to side through the center. The gas thus obtained was transferred to a Bonnier-Mangin apparatus described by Aubert (*1*) and Grafe (*4, p. 377*). The absorption of carbon dioxide was accomplished by using a solution of potassium hydroxide, while oxygen was absorbed with an alkaline solution of pyrogallol. The gas remaining after the carbon dioxide and oxygen had been absorbed was assumed to be nitrogen.

The results obtained are shown in Table 3. Most noticeable is the fact that in the Irish Cobbler and Green Mountain varieties a higher content of CO_2 was found at 36° F. than at any other temperature, whereas in the Triumph variety the CO_2 content was highest at 32°. With a relatively large amount of CO_2 a correspondingly small volume of oxygen was usually found. These results apparently can not be correlated with the relative respiration rate or sugar content at the different temperatures; furthermore, the internal gas composition in all varieties fluctuated with changes in temperature in such a way as to suggest a need for further study in order to determine what relations may exist between the internal atmospheric composition and other physiological reactions.

FURTHER CHANGES IN IRISH COBBLER AND GREEN MOUNTAIN POTATOES AT 60° F., AFTER STORAGE AT 32°

The results shown in Table 1 indicate that potatoes stored at a constant temperature of 32° F. increase in sugar content considerably during storage. The maximum sugar content for all varieties was

found on March 24. On this date Irish Cobbler and Green Mountain potatoes from the lots already described were transferred and held at a constant temperature of 60° for an intensive study of the changes in their sugar content, rate of respiration, and internal atmospheric composition.

SUGAR CONTENT

Samples for sugar determinations were taken on the fourth day after the transfer to storage at 60° F. and frequently thereafter for a period of 20 days, when evidence of sprouting became apparent and further sampling was discontinued. The results presented in Table 4 show a rather uniform decrease in sugars up to the time sampling was discontinued. At this time the total sugar present in the Irish Cobbler variety was still somewhat higher than that found in the fall before the storage experiments started; whereas, in the Green Mountain variety, when sampling was discontinued on April 10, both reducing and total sugars were less than when the experiment started. These data are in agreement with results already well known from earlier investigations on potato storage.

TABLE 4.—*Changes in sugar content of potatoes after being stored at 32° F. from November 5 to March 24 and then transferred to storage at 60°*

Sugars expressed as percentage of dextrose based on fresh weight

Date	Irish Cobbler			Green Mountain		
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar
Nov. 5 [a]	0.91	0.27	1.18	1.99	0.27	2.26
Mar. 24	4.42	1.45	5.87	4.87	2.41	7.28
Mar. 28	3.47	1.00	4.47	3.34	1.26	4.60
Mar. 30	3.15	.83	3.98	2.66	.92	3.58
Apr. 2	2.50	.68	3.18	2.50	.77	3.27
Apr. 6	1.90	.47	2.37	2.30	.57	2.87
Apr. 10	1.81	.32	2.13	1.63	.37	2.00
Apr. 17	1.61	.30	1.91			

[a] Before storage.

RESPIRATION

After the potatoes had been transferred to storage at 60° F. and had warmed up to the air temperature (in about 24 hours), samples of approximately 3 kg each were put in the respiration apparatus already described, and the progressive rate of respiration was determined for both varieties by separate runs of varying duration, as shown in Table 5. A previous set of respiration tests was made on Irish Cobbler potatoes after they had been held at 32° only from November 7 to January 17 before being transferred to storage at 60°. It should be noted that sprouting became apparent about nine days after this transfer. These potatoes had been harvested long enough to have passed through their rest period and would naturally sprout at this comparatively high temperature. The results are shown in detail in Table 5.

In both varieties the respiration rate, as indicated by the CO_2 given off per kilogram per hour, showed a tendency to increase during the first few days and then to decrease gradually until it nearly reached that of potatoes held continuously in storage at 60° F. (Table 2.)

This was in general accord with the results reported by Kimbrough (*6*).

TABLE 5.—*Respiration at 60° F. of two lots of Irish Cobbler and one lot of Green Mountain potatoes, after storage at 32° from November 7 to January 17, November 7 to March 24, and November 7 to March 24, respectively*

Run No.	Respiration test at 60° F. after indicated period of storage at 32°					
	Irish cobbler				Green Mountain	
	Nov. 7 to Jan 17 [a]		Nov. 7 to Mar. 24. [b]		Nov. 7 to Mar. 24 [b]	
	CO_2 per kilogram per hour	Time in 60°	CO_2 per kilogram per hour	Time in 60°	CO_2 per kilogram per hour	Time in 60°
	Mg	*Hours*	*Mg*	*Hours*	*Mg*	*Hours*
1	17.7	49	13.7	66	10.65	66
2	26.5	92	15.6	139	12.80	139
3	25.8	139	14.4	235	12.20	235
4	17.8	217	18.2	310	14.20	310
5	13.7	289	16.8	478	14.00	478
6	10.2	406	12.4	526	8.80	526
7	9.2	601				
8	6.0	765				

[a] Test was begun Jan. 18 on sample weighing approximately 3 kg.
[b] Test was begun Mar. 25 on sample weighing approximately 3 kg.

In the lot of Irish Cobbler potatoes placed at 60° F. on January 17 the increasing rate of respiration for the first few days was followed by a gradual diminution. This increase in respiration in potatoes high in sugar content, removed from a low temperature, is consistent with the results reported by Müller-Thurgau (*9*). However, the increase in rate lasted longer than he reported (one and one-half to two days), although this may be accounted for by the fact that his investigation was carried on at 68° F. (20° C.) instead of at 60°. The respiration rate in comparable lots of potatoes, that is, potatoes from the same general lots, when harvested and held constantly at 60°, was not determined as late as March 24, because sprouting had already begun by this time. Table 2 shows that on January 17, the respiration rates, as denoted by the CO_2 given off, were 10.40 and 5.80 for the Irish Cobbler and Green Mountain potatoes, respectively. These are less than the respiration rate (17.7) found during the first run in the Irish Cobbler potatoes transferred from storage at 32° to storage at 60° on January 17, and also less than that found in either of the varieties transferred on March 24, when the respiration rates were 13.7 and 10.65, respectively. These values show an increased respiration rate in potatoes moved from storage at 32° to storage at 60°, as reported by previous workers. Comparing the simultaneous respiration activity of potatoes remaining in storage at 32° with that of the lots transferred on March 24 to storage at 60°, the rates were 3.27 and 2.99 (see Table 2) for the Irish Cobbler and Green Mountain varieties, respectively, at 32°, as against 13.7 and 10.65 in the first runs of these varieties after 66 hours at 60°.

COMPOSITION OF INTERNAL ATMOSPHERE

Simultaneously with studies on the changes in sugar content and respiration rate, as reported, changes in internal atmosphere of the tubers were studied. The results are shown in Table 6. Here a marked increase in CO_2 content over the amount present when the potatoes were transferred was found in both varieties on the second day at 60° F. This comparatively high CO_2 content of the internal atmosphere of the tubers persisted for about six days and was correlated with a higher rate of respiration during this period. There was a general, but not always consistent, tendency for the oxygen to decrease as the CO_2 increased.

TABLE 6.—*Change in percentage composition of the internal atmosphere of Irish Cobbler and Green Mountain potatoes at 60° F., after storage at 32° from November 7 to March 24*

Number of days at 60° F.	Irish Cobbler			Green Mountain		
	CO_2	O_2	N	CO_2	O_2	N
0	11.4	19.2	69.4	4.7	47.5	47.8
2	32.5	8.5	59.0	23.6	16.1	60.3
4	29.7	11.1	59.2	18.1	19.9	62.0
6	30.5	26.3	43.2	24.8	18.3	56.9
9	20.6	15.6	63.8	16.8	15.2	68.0
13	17.5	18.1	64.4	17.7	14.6	67.7
17	14.7	16.2	69.1	15.0	16.7	68.3
24	12.2	18.5	69.3			

CHANGES IN FROZEN POTATOES

Irish Cobbler and Green Mountain potatoes of the same general lot as already described were taken from continuous storage at 40° F. on March 11 and exposed to a temperature of 22°. They were spread out in a single layer for three hours to bring their internal temperatures to the freezing point or below, and then all were sharply disturbed ("inoculated") by being quickly tossed in a basket and then poured out in a layer as before, each potato being allowed to drop a distance of 3 or 4 inches. This operation was to start simultaneous freezing in order to have approximately the same amount of injury in all specimens when removed. After "inoculation" the tubers were left undisturbed for 2 hours, when they were gathered up and allowed to thaw out at 40° for 24 hours. An examination at this time by cutting various tubers showed mild freezing injury of the net or ring type.

SUGAR CONTENT

After warming at 40° F. for 24 hours, ½-bushel lots of the tubers that had been frozen and similar lots of unfrozen specimens from constant 40° storage were put in storage at 32°, 40°, 50°, 60°, 70°, and 85° for one month. Sugar determinations were than made on samples of about 10 tubers from all lots. (Table 7.) All the analyzed tubers from the lots that had been frozen showed necrosis. Initial sugar determinations were not made, for it was assumed that since the frozen potatoes had remained at the low temperature for only five hours they would show no difference from the unfrozen ones.

TABLE 7.—*Sugar-content changes in frozen and unfrozen potato varieties after one month of storage at various temperatures*

[Sugars expressed as percentage of dextrose based on fresh weight]

Storage temperature (°F.)	Frozen [a]			Unfrozen		
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar
IRISH COBBLER						
32	2.78	1.15	3.93	2.52	0.70	3.22
40	1.35	.34	1.69	1.52	.26	1.78
50	1.00	.26	1.26	.94	.24	1.18
60	.87	.11	.98	.79	.26	1.05
70	.72	.86	1.58	.53	.82	1.35
85	.53	.34	.87	.39	.09	.48
GREEN MOUNTAIN						
32	2.98	1.80	4.78	2.98	1.13	4.11
40	1.65	.41	2.06	1.69	.48	2.17
50	1.13	.20	1.33	1.20	.23	1.43
60	1.15	.20	1.35	.89	.20	1.09
70	.75	.40	1.15	.57	.41	.98
85	.83	.84	1.67	.44	.11	.55

[a] The frozen potatoes were held at a temperature of 22° F. for 3 hours, inoculated by being jarred, and then allowed to freeze for 2 hours, after which they were held at 40° for 24 hours to warm up.

These results did not consistently prove or disprove the common belief that frozen potatoes are sweeter than unfrozen ones. At some holding temperatures the sugar content of the frozen potatoes was somewhat higher than that of unfrozen potatoes, and at other temperatures the reverse was true; moreover, the same relations did not hold for both varieties. Much of this lack of correlation can be explained by the possibility that different degrees of freezing occurred and caused variation among individual specimens. The inference that in most cases there was a slightly higher sugar content in potatoes that had been frozen is consistent with the slightly higher rate of respiration shown later.

RESPIRATION

Approximately 3-kg duplicate lots of frozen Irish Cobbler potatoes from the lot described were transferred to storage at 60° F., after they had thawed out at 40° for 24 hours, and were left for about 12 hours to reach this temperature, along with similar lots from the constant storage at 40°. These lots of potatoes were then put in the respiration apparatus and the comparative rates of respiration of frozen and unfrozen potatoes determined in three runs, as shown in Table 8. In every run a somewhat higher respiration rate was found in the frozen potatoes than in the unfrozen ones from the same general source.

TABLE 8.—*Respiration at 60° F. of frozen and unfrozen Irish Cobbler potatoes*

Run No.	Frozen [a]		Unfrozen	
	CO_2 per kilogram per hour	Duration of run	CO_2 per kilogram per hour	Duration of run
	Mg	*Hours*	*Mg*	*Hours*
1	16.6	72	9.7	72
2	15.2	72	12.1	72
3	11.1	120	9.3	120

[a] The frozen potatoes were held at 22° for 3 hours, inoculated by being jarred, and then allowed to freeze for 2 hours, after which these, as well as the unfrozen potatoes, were placed at 60° to warm up overnight

SUMMARY

Large representative lots of Irish Cobbler, Green Mountain, and Triumph potatoes were stored continuously at 32°, 36°, 40°, 50°, 60°, and 70° F. from November 5 until March 24, and periodic determinations were made of sugar content and respiration rate at these temperatures. Analyses of the internal or intercellular atmosphere were also made after holding the potatoes in storage at these temperatures from November 5 until February 18.

At 32° F. the sugar content steadily increased in all varieties until March 24. After this, further analyses were not made until June 1, when a slight decrease was shown. At 36° there was a marked increase in sugar, but to a less extent than at 32°. At 40° the periodic results were fluctuating, but on the whole there was a slight increase in sugar. At 50°, 60°, and 70° the sugar content decreased steadily in proportion as the temperature increased.

Respiration of Irish Cobbler and Green Mountain potatoes increased at successively higher temperatures above 40° F. Respiration at 36° was more rapid than at 40° or 32°. In general the respiration rate decreased as the storage period increased.

The CO_2 content of the internal atmosphere of Irish Cobbler and Green Mountain tubers was greatest at 36° F., and that of the Triumph variety was greatest at 32°. At the other temperatures no apparent correlation existed between the internal atmospheric composition and the temperature.

After Irish Cobbler and Green Mountain potatoes had been in storage at 32° F. until March 24, some of each of the varieties were removed and held at 60°; sugar content, respiration rate, and composition of the internal atmosphere were then determined at frequent intervals.

The sugar content steadily and rapidly decreased, while the rate of respiration at first increased and then decreased. The CO_2 content of the internal atmosphere increased rapidly during the first two days and remained high for about six to nine days before decreasing.

Irish Cobbler and Green Mountain potatoes, after being in storage at 40° F. until March 11, were slightly frozen at 22° for five hours. They were then thawed at 40°, divided, and stored at 32°, 40°, 50°, 60°, 70°, and 85°, along with unfrozen lots from the same temperature. After a month's storage sugar determinations were made. At most temperatures the potatoes that had been frozen showed a somewhat higher sugar content than did the unfrozen potatoes.

Samples of the frozen potatoes, after thawing for 24 hours at 40° F., together with unfrozen potatoes from storage at 40°, were stored at 60° for 24 hours so as to allow their temperature to come to room temperature. The rate of respiration was then determined in both lots. A somewhat higher rate was found in the frozen potatoes.

LITERATURE CITED

(1) AUBERT, E.
1891. NOUVEL APPAREIL DE MM. G. BONNIER ET L. MANGIN POUR L'ANALYSE DES GAZ. Rev. Gén. Bot. 3:97–104, illus.

(2) BENNETT, J. P., and BARTHOLOMEW, E. T.
1924. THE RESPIRATION OF POTATO TUBERS IN RELATION TO THE OCCURRENCE OF BLACKHEART. Calif. Agr. Expt. Sta. Tech. Paper 14, 35 p., illus.

(3) GORE, H. C.
1911. STUDIES ON FRUIT RESPIRATION. I. THE EFFECT OF TEMPERATURE ON THE RESPIRATION OF FRUITS. II. THE EFFECT OF PICKING ON THE RATE OF EVOLUTION OF CARBON DIOXID BY PEACHES. III. THE RATE OF ACCUMULATION OF HEAT IN THE RESPIRATION OF FRUIT UNDER ADIABATIC CONDITIONS. U. S. Dept. Agr., Bur. Chem. Bul. 142, 40 p., illus.

(4) GRAFE, V.
1914. ERNAHRUNGSPHYSIOLOGISCHES PRAKTIKUM DER HÖHEREN PFLANZEN. 494 p. Berlin.

(5) HOPKINS, E. F.
1924. RELATION OF LOW TEMPERATURES TO RESPIRATION AND CARBOHYDRATE CHANGES IN POTATO TUBERS. Bot. Gaz. 78:[311]–325, illus.

(6) KIMBROUGH, W. D.
1925. A STUDY OF RESPIRATION IN POTATOES WITH SPECIAL REFERENCE TO STORAGE AND TRANSPORTATION. Md. Agr. Expt. Sta. Bul. 276, p. [51]–72, illus.

(7) MAGNESS, J. R.
1920. COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES. Bot. Gaz. 70:308–316, illus.

(8) ——— and DIEHL, H. C.
1924. PHYSIOLOGICAL STUDIES OF APPLES IN STORAGE. Jour. Agr. Research 27:1–38, illus.

(9) MÜLLER–THURGAU, H.
1880–1886. UEBER DAS GEFRIEREN UND ERFRIEREN DER PFLANZEN. Landw. Jahrb. 9:[133]–189, 1880; 15:[453]–610, illus. 1886.

APPARENT DIGESTIBILITY OF, AND NITROGEN, CALCIUM, AND PHOSPORUS BALANCE OF DAIRY HEIFERS ON, ARTIFICALLY DRIED PASTURE HERBAGE [1]

By R. E. HODGSON, *Agent, Bureau of Dairy Industry, United States Department of Agriculture, Dairy Husbandman, Western Washington Experiment Station, and Assistant in Dairy Husbandry, Washington Agricultural Experiment Station,* and J. C. KNOTT, *Superintendent of Official Testing, Washington Agricultural Experiment Station* [2]

INTRODUCTION AND REVIEW OF PREVIOUS INVESTIGATIONS

The value of an intensive system of pasture management in increasing the yield and nutritive value of pasture herbage has been amply demonstrated by various investigators.

Results obtained by Grunder [3] in a study of the yield and chemical composition of mixed pasture grasses and clovers show that under a system of biweekly cuttings the dry matter of the herbage possessed an average percentage composition of 26.28 crude protein, 17.52 crude fiber, 40.72 nitrogen-free extract, 3.31 ether extract, 12.17 total ash, 0.57 phosphorus, and 0.66 calcium.

Ellenberger (*1*) [4] and others reported that the dry matter of ordinary pasture grass in Vermont had an average composition of 19.05 per cent crude protein, 17.87 per cent crude fiber, 48.31 per cent nitrogen-free extract, 0.854 per cent calcium, and 0.270 per cent phosphorus. They concluded that quantity rather than quality was the dominating factor affecting carrying capacity of closely grazed pastures.

Woodman and his associates (*7, 8, 9, 10, 11, 12, 13*) reported, from an extensive study of the nutritive value of pastures, that young pasture grass obtained under a weekly, biweekly, triweekly, or monthly system of cutting was, in terms of its digestible nutrient, a "watered concentrate" rather than a roughage. Seventy-two per cent of the organic matter as well as 80 per cent of the small amount of fiber present in the grass was digestible. The immature herbage contained approximately 20 per cent digestible crude protein.

The proper preservation of large amounts of this highly nutritious feed without affecting its feeding value is a matter of much interest at the present time. With the advent of the commercial hay drier, it seems possible that immature pasture herbage possessing a highly digestible protein content might be harvested, artificially dried, and fed to dairy cattle as a protein-rich concentrate. Reports of the digestibility of artificially dried grasses would seem to indicate that the process of drying does not greatly depress their feeding value.

Woodman and others (*11*) have shown that young grass does not suffer any appreciable depression in digestibility when artificially

[1] Received for publication February 16, 1932, issued November 1932. Scientific Paper No. 218, College of Agriculture and Experiment Station, State College of Washington.

[2] The writers are indebted to Harold Gerritz, who was responsible for all the analytical work involved, and to M. S. Grunder, under whose direction the herbage was collected and preserved.

[3] Unpublished data obtained from M. S. Grunder, agronomist, Western Washington Experiment Station Puyallup, Wash.

[4] Reference is made by number (italic) to Literature Cited. p. 562.

dried at the temperature of steam or by direct heat in a kiln. The digestibility coefficients of the total organic matter and of the crude protein portion of the kiln-dried grass were 79.44 and 78.24, respectively.

Watson (*6*) has presented data showing that sheep on an exclusive diet of artificially dried pasture grass were able to maintain their live weight. The digestibility coefficients of the organic matter and crude protein of the dried grass were 76.36 and 76.35, respectively. The sheep maintained positive nitrogen, calcium, phosphorus, and potassium balances throughout the trial.

Watson (*6*) fed artificially dried pasture grass to milking dairy cows and found it to be very palatable. It proved to produce the quantity of milk for which the nutrients it supplied were theoretically capable.

During the winter of 1931 the apparent digestibility of artificially dried pasture herbage was studied at this station. Dairy heifers were used as the experimental animals. In conjunction with this work, it was thought advisable to determine the balance of nitrogen, calcium, and phosphorus of heifers on an exclusive diet of artificially dried pasture herbage.

EXPERIMENTAL PROCEDURE

The artificially dried pasture herbage was obtained from plots of pure stands of the following grasses and clovers: English ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), orchard grass (*Dactylis glomerata*), rough-stalked meadow grass (*Poa trivialis*), Kentucky bluegrass (*P. pratensis*), annual bluegrass (*P. annua*), meadow fescue (*Festuca elatior*), creeping bent (*Agrostis stolonifera*), ladino clover (*Trifolium repens latum*), alsike clover (*T. hybridum*), and white clover (*T. repens*). These grasses and clovers were cut with a lawn mower at biweekly intervals and dried in an experimental drier and mixed. The material was cut into approximately 3-inch lengths. The predominating plants in the resulting mixture were English ryegrass and Italian ryegrass.

Drying was done in a small batch drier. Heated air passing through the oven at from 100° to 200° F. dried the material to approximately 5 per cent moisture in about 12 hours. The dried herbage retained its green color and possessed a pleasing aroma.

The usual precautions necessary in conducting a digestibility and mineral-balance study as outlined by Forbes and Grindley (*3, p. 17–27*) were observed throughout the experiment. The experimental animals were three Holstein heifers, approximately 2 years old and weighing on an average about 950 pounds each. They were gradually changed to the experimental ration prior to going into the digestion stalls at the beginning of the preliminary period.

An amount of artificially dried pasture herbage sufficient to last throughout the experiment was spread out on a tight floor, thoroughly mixed, and divided into three piles. The individual feeds for the entire period were weighed out into paper bags; a part of each feed being taken from each of the three piles. After five portions had been weighed, a small amount of the feed was taken from each of the three piles to form a composite sample for chemical analysis.

The daily ration used was calculated to satisfy the total digestible nutrient requirements of growing dairy animals as suggested by Fitch

and Lush (*2*). Watson's (*6*) digestibility figures for artificially dried grass were used as a basis for calculating these rations. The feed proved to be very palatable, and throughout the experiment all that was offered the animals was readily eaten. Distilled water was available to the animals at all times. Three-fourths of an ounce of chemically pure sodium chloride was fed daily.

The preliminary feeding period lasted 10 days, the collection period 15 days. The experimental day began at 3.00 p. m., at which time the live weights of the heifers were recorded. Feeding was done at 4.30 p. m. and 5.30 a. m. The animals were exercised at 10 a. m. daily.

The urine and feces were collected by three attendants who worked in 8-hour shifts. The urine was caught in suitable containers and placed in bottles. The 24-hour sample from each heifer was weighed, thoroughly mixed, and an aliquot of 10 per cent was taken and analyzed daily for nitrogen, calcium, and phosphorus.

The feces were caught in pails and transferred to covered, galvanized-iron cans. At the close of each experimental day the feces were weighed and mixed, and a representative 5 per cent sample was placed in a friction-top lard pail, sealed, and stored in a refrigerator at $-10°$ F. At the close of the collection period, the aliquot samples representing the total feces collected from each animal were mixed, ground, remixed, and sampled for chemical analysis.

The analyses of the feed, urine, and feces were carried out by the usual standard methods. Determinations of iron and silica were made on the feed and feces for the purpose of comparing the apparent digestibility of the various constituents of the feed as determined by the usual method with the modified procedure suggested by Gallup and Kuhlman (*4*).

EXPERIMENTAL DATA

The results of this investigation are presented in four tables. The amount of artificially dried herbage consumed and the feces and urine voided during the experimental period are given in Table 1. Table 2 shows the percentage composition of the feed, feces, and urine. In Table 3 are shown the quantities of the various feed constituents ingested, voided, and retained by each heifer during the 15-day collection period. This table also shows the apparent digestibility in percentage of the feed constituents as determined under the conditions of this experiment.

TABLE 1.—*Feed consumed and feces and urine voided (grams) during 15-day metabolism trial*

Heifer No.	Feed consumed	Feces voided	Urine voided
107	102, 270	125, 204	227, 623
108	102, 270	155, 084	203, 795
109	102, 270	133, 090	247, 918

TABLE 2.—*Percentage composition of feed, feces, and urine*

[Dry-matter basis]

Heifer No. and material analyzed	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash	Nitrogen	Calcium	Phosphorus
107:									
Feces	21.71	18.81	30.72	8.14	15.73	23.60	3.01	2.39	1.87
Urine							1.174	.002	.012
108:									
Feces	19.66	18.31	30.87	8.24	15.16	23.43	2.93	3.29	1.87
Urine							1.270	.004	.005
109:									
Feces	20.99	20.12	30.66	8.81	14.86	22.80	3.22	2.32	1.84
Urine							1.032	.002	.009
All 3 heifers: Feed	86.05	24.64	39.19	3.50	18.09	11.18	3.94	.78	.65

TABLE 3.—*Apparent digestibility of artificially dried pasture herbage fed to each animal*

HEIFER 107

Item	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Ingested grams	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided do	27,181.79	5,112.89	8,350.25	2,212.60	4,275.70	6,414.90
Digested do	60,821.55	16,571.13	26,138.26	867.52	11,644.10	3,423.87
Digested per cent	69.11	76.42	75.79	28.16	73.14	34.80

HEIFER 108

Ingested grams	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided do	30,489.51	5,582.63	9,412.11	2,542.82	4,622.21	7,143.69
Digested do	57,513.83	16,101.39	25,076.40	537.30	11,297.59	2,695.08
Digested per cent	65.35	74.25	72.71	17.44	70.97	27.39

HEIFER 109

Ingested grams	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided do	27,935.59	5,620.64	8,565.05	2,461.12	4,151.23	6,369.31
Digested do	60,067.75	16,063.38	25,923.46	619.00	11,768.57	3,469.46
Digested per cent	68.26	74.08	75.16	20.10	73.92	35.26
Average percentage digested by all 3 heifers	67.57	74.92	74.55	21.90	72.68	32.48

The digestibility coefficients in all cases, with the exception of the ether extract and ash, are in close agreement for the three heifers. The average digestibility coefficient of 74.92 for crude protein gave this artificially dried pasture herbage a digestible crude-protein content of more than 18 per cent, which compares favorably with some of the higher protein concentrates.

The dried herbage had a digestible carbohydrate equivalent of 45.63 per cent and a total digestible nutrient content of 64.74 per cent. The high digestibility of the crude fiber contributes materially to the feeding value of this feed.

The average digestibility coefficient of 21.9 for the ether extract as obtained in this experiment is considerably lower than the 59.6 per cent obtained by Watson (*5*) or the 70.6 per cent obtained by Woodman and his associates (7). Not only was the digestibility

of the ether extract low in this experiment, but considerable variation was shown by individual animals. It is possible that the method of drying the feed may have depressed the digestibility of the ether extract.

Heifer 108 had the lowest coefficient of digestibility for each constituent except protein. This heifer was in much better condition than either heifer 107 or 109. The digestibility of artificially dried pasture herbage as determined in this experiment with dairy heifers was uniformly lower than that obtained by Watson (*6*) or by Woodman et al. (*11*) with sheep.

The average daily balance of nitrogen, calcium, and phosphorus for each of the three heifers is given in Table 4. A slight negative

TABLE 4.—*Average daily nitrogen, calcium, and phosphorus balances (in grams) for the three heifers during 15-day metabolism trial*

Heifer No.	Nitrogen			Calcium			Phosphorus		
	Fed	Eliminated	Balance	Fed	Eliminated	Balance	Fed	Eliminated	Balance
107	231.16	232.68	−1.52	45.57	43.67	+1.90	38.25	35.64	+2.61
108	231.16	232.12	−.96	45.57	67.45	−21.88	38.25	38.74	−.49
109	231.16	230.58	+.58	45.57	43.53	+2.04	38.25	35.78	+2.47
Average balance			−.63			−5.98			+1.53

average daily nitrogen balance may indicate that some of the protein ingested was used for the production of energy. According to the apparent digestibility figures obtained in this experiment, the ration fed these animals furnished an excess of protein but did not furnish sufficient energy. In spite of the insufficient supply of energy in the ration, each of the three heifers made substantial gains in live weight throughout the collection period, which would further indicate the utilization of protein material for the production of energy.

Heifer 107 had an average initial live weight of 940 pounds and an average final weight of 952.8 pounds, indicating a gain of 12.8 pounds for the 15-day collection period. Heifer 108 had an average initial live weight of 1,023.6 pounds and an average final weight of 1,048.6 pounds, indicating a gain of 25 pounds during the collection period. Heifer 109 weighed 875.8 pounds initially, 897.2 pounds finally, and gained 21.4 pounds. The average daily gain in live weight per animal was 1.32 pounds.

Heifer 109 showed a slight positive daily nitrogen balance. This was the smallest and poorest-conditioned animal of the three. It is probable that the energy content of the ration more nearly supplied the energy requirements of this heifer and, therefore, not so much of the protein digested was used for the production of energy.

Heifers 107 and 109 showed a positive average daily calcium balance of 1.90 g and 2.04 g, respectively, while heifer 108 had a negative average daily calcium balance of 21.88 g. Likewise heifers 107 and 109 had a positive daily phosphorus balance of 2.61 g and 1.53 g, while heifer 108 had a negative daily balance of 0.49 g. The average daily intake of calcium per animal was 45.57 g and of phosphorus 38.25 g, which gives a calcium-phosphorus ratio of 1.2:1.

Lindsey and his associates (*5*) were able to secure normal growth of Holstein heifers in their third year with an average daily intake of 2.5 g of calcium per 100 pounds live weight. They found that the calcium and phosphorus was retained at the approximate ratio of 2 to 1. On the basis of the above findings, the heifers in this experiment were furnished with an abundance of both calcium and phosphorus. However, the retention ratio with the two animals having positive calcium and phosphorus balances was about 0.78 calcium to 1 of phosphorus. With a low calcium and phosphorus retention in the case of two animals and a negative balance in the case of the third, together with an unusual calcium and phosphorus retention ratio, there are apparently some factors that have interfered with normal retention of these constituents in this experiment.

SUMMARY

Metabolism experiments with three 2-year-old Holstein heifers were conducted to determine the apparent digestibility of artificially dried pasture herbage and to determine the nitrogen, calcium, and phosphorus balances of these heifers on an exclusive diet of dried herbage.

The crude protein, which constituted 24.64 per cent of the dry matter of the artificially dried pasture herbage, had an average digestibility coefficient of 74.92 per cent.

The average digestibility of the nitrogen-free extract was 74.55 per cent.

The digestibility of the ether extract was rather low, the average for the three heifers being only 21.90 per cent. This low digestibility of the ether extract may possibly have been caused by the drying process.

The crude fiber was found to be 72.68 per cent digestible. The digestible crude fiber contributes much to the feeding value of artificially dried pasture herbage.

The nitrogen balances were negative in two of the three cases with an average negative balance of 0.63 g daily.

Two heifers showed a positive daily calcium balance of 1.90 g and 2.04 g, respectively, while the third had a negative daily balance of 21.88 g. This latter animal also showed a negative phosphorus balance of 0.49 g daily, while the other two had positive balances of 2.61 g and 1.53 g, respectively.

The average daily intake of calcium was 45.57 g and of phosphorus 38.25 g, which gives a calcium-phosphorus ratio of 1.2:1.

LITERATURE CITED

(1) Ellenberger, H. B., Newlander, J. A., and Jones, C. H.
1929. yield and composition of pasture grass. Vt. Agr. Expt. Sta. Bul. 295, 68 p., illus.

(2) Fitch, J. B., and Lush, R. L.
1931. an interpretation of the feeding standards for growing dairy cattle. Jour. Dairy Sci. 14: 116–124, illus.

(3) Forbes, E. B., and Grindley, H. S.
1923. on the formulation of methods of experimentation in animal production. Bul. Natl. Research Council 6 (pt. 2, no. 33), 54 p.

(4) Gallup, W. D., and Kuhlman, A. H.
1931. a preliminary study of the determination of the apparent digestibility of protein by modified procedures. Jour. Agr. Research 42:665–669.

(5) LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R.
1931. THE CALCIUM REQUIREMENTS OF DAIRY HEIFERS. Jour. Agr. Research 42:883–896, illus.

(6) WATSON, S. J.
1931. INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT. IV. THE DIGESTIBILITY AND FEEDING VALUE OF ARTIFICIALLY DRIED GRASS. Jour. Agr. Sci. [England] 21: 414–424, illus.

(7) WOODMAN, H. E., BLUNT, D. L., and STEWART, J.
1926. NUTRITIVE VALUE OF PASTURE. I. SEASONAL VARIATIONS IN THE PRODUCTIVITY, BOTANICAL AND CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF MEDIUM PASTURAGE ON A LIGHT SANDY SOIL. Jour. Agr. Sci. [England] 16:[205]–274, illus.

(8) ——— BLUNT, D. L., and STEWART, J.
1927. NUTRITIVE VALUE OF PASTURE. II. SEASONAL VARIATIONS IN THE PRODUCTIVITY, BOTANICAL AND CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF PASTURAGE ON A HEAVY CLAY SOIL. Jour. Agr. Sci. [England] 17:[209]–263, illus.

(9) ——— NORMAN, D. B., and BEE, J. W.
1928. NUTRITIVE VALUE OF PASTURE. III. INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART I). Jour. Agr. Sci. [England] 18: [266]–294.

(10) ——— NORMAN, D. B., and BEE, J. W.
1929. NUTRITIVE VALUE OF PASTURE. IV. THE INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART II). Jour. Agr. Sci. [England] 19:[236]–265.

(11) ——— BEE, J. W., and GRIFFITH, G.
1930. NUTRITIVE VALUE OF PASTURE. V. PASTURE GRASS CONSERVATION: THE INFLUENCE OF ARTIFICIAL DRYING ON THE DIGESTIBILITY OF PASTURE HERBAGE. Jour. Agr. Sci. [England] 20:[53]–62.

(12) ——— and EVANS, R. E.
1930. NUTRITIVE VALUE OF PASTURE. VI. THE UTILIZATION BY SHEEP OF MINERAL-DEFICIENT HERBAGE. Jour. Agr. Sci. [England] 20: [586]–[617].

(13) ——— NORMAN, D. B., and FRENCH, M. H.
1931. NUTRITIVE VALUE OF PASTURE. VII. THE INFLUENCE OF INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART III). Jour. Agr. Sci. [England] 21:[267]–[323].

AN EMPIRICAL TEST OF THE APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES[1]

By JAY L. LUSH

Head, Animal Breeding Subsection, Animal Husbandry Section, Iowa Agricultural Experiment Station

INTRODUCTION

In 1925 [2] Wright and McPhee reported a sampling method for calculating inbreeding coefficients and relationship coefficients from random ancestral lines of livestock pedigrees. In applying this method to a study of the genetic history of the Rambouillet breed of sheep the writer had occasion to make some empirical tests of its reliability.

PLAN OF STUDY

The study involved a series of samples, each consisting of 400 pedigrees of sheep registered as having been born in a certain year. The pedigrees in each sample were systematically selected at regular intervals in the appropriate volume of the flock book, without regard to breeder, geography, or individual merit. From each of these 400 pedigrees two random lines of ancestry, one from the sire and one from the dam, were traced back to the beginning of the flock book, each sequence of sires or dams being determined at random. The samples from two years (1916 and 1926) were duplicated to make the findings of the study more reliable and to test certain apparent discrepancies in the genetic trends from period to period. A study of the differences between the two 1916 samples and the differences between the two 1926 samples is the basis of the present study. In taking the duplicate samples, animals already used in the first sample were avoided and, as far as possible, animals for the second sample were taken from pages other than those from which the first sample animals had been taken.

The second 1916 sample did not agree at all with the first in inbreeding coefficients, although it did agree fairly well in relationship coefficients. The difference in inbreeding coefficients was a little more than four times its probable error. This led to a test to determine whether the difference lay in the method of sampling the breed or in the method of sampling the ancestral lines of the pedigrees after the pedigrees had been selected. Using the very same animals selected in the first 1916 sample, the writer traced back new random lines from the sires and dams. The results were in almost identical agreement with the findings of the second sample rather than with the random lines first run from the first sample. To complete this test, new random lines were traced back from the sires and dams of

[1] Received for publication Feb. 22, 1932; issued November, 1932. Journal Paper No. B38 of the Iowa Agricultural Experiment Station.

[2] WRIGHT, S. and MCPHEE, H. C. AN APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES. Jour. Agr. Research 31:377-383. 1925.

the animals in the second sample of 400 pedigrees. Again there resulted almost identical agreement with the first sampling of those pedigrees and with the second sampling of the first 1916 pedigrees. In the second trials of the two 1916 samples only the inbreeding coefficients were studied.

INBREEDING COEFFICIENTS

The results of the tests are shown in Table 1. One of the four 1916 trials was so far out of line with the other three that it demanded an explanation. Only seven different ancestors furnished ties in both of the 1926 samples. Four different ancestors each furnished one or more ties for each of the four 1916 trials, 6 ancestors furnished ties in both trials of the second 1916 sample, and 17 of the 28 different ancestors that furnished ties in the second trial of the first 1916 sample had also furnished ties in the first trial of that sample.

TABLE 1.—*Agreement between duplicate samples, each of four hundred 2-column pedigrees of Rambouillet sheep*

Item	Sheep born in 1926		Sheep born in 1916			
			First sample		Second sample	
	First sample	Second sample	First trial	Second trial	First trial	Second trial
Inbreeding:						
Ties found	41	44	53	29	29	29
Number of different ancestors on which the ties occurred	38	41	41	28	28	27
Coefficient	5.3±0.5	5.6±0.5	6.8±0.6	3.7±0.4	3.7±0.4	3.7±0.4
Inter se relationship:						
Ties found	11	10	9		12	
Coefficient	2.7±0.5	2.5±0.5	2.1±0.5		3.0±0.6	
Relationship to individual animals:						
Registry No.—						
328	2.3	1.4	2.5		3.5	
922	4.1	5.4	4.8		5.9	
923	2.8	3.6	1.9		2.3	
952	4.4	4.6	5.0		4.8	
961	5.9	8.2	5.3		6.9	
1230	1.7	2.3	2.4		1.4	
1245	.9	1.3	1.2		1.1	
1261	2.0	3.0	3.4		1.5	
1268	2.1	3.2	3.2		2.0	
2937	3.6	4.3	5.4		5.4	
2938	.5	.6	.5		.4	
2940	2.3	2.6	2.4		2.6	
13402	5.7	6.9	5.4		6.2	
13405	3.8	1.6	3.0		2.5	
13406	3.4	2.4	3.4		3.9	
13411	4.7	3.7	4.6		5.2	
13420	4.4	5.7	6.8		5.4	
13435	5.4	6.6	7.9		6.4	
13436	3.7	5.0	8.5		6.1	
13442	6.6	6.2	7.9		8.1	
13454	5.6	6.2	6.0		6.1	
17519	6.3	4.6	6.2		6.2	
20079	3.6	2.4	2.7		1.1	
29311	4.9	6.4				
29516	6.1	5.8	6.5		6.8	
31522	3.8	2.4				
34671	5.0	5.6				
37853	5.9	4.9				
39516	2.8	2.8				
39636	6.9	8.6	1.2		.6	
52810	5.5	5.1	6.2		5.5	
69456	5.0	6.9	5.5		6.0	
98869	6.7	7.2				

The random sequences were obtained in these studies by tossing a penny for each sequence instead of establishing a chain of some thousand sequences and following that chain through again and again, as was suggested by Wright and McPhee. It seems certain that the discrepancy in the first trial of the first 1916 sample arose on account of a systematic error that inadvertently crept in through the use of this method. When a new sequence was desired, a penny was drawn from the box and laid on the table and the sire or dam was selected according to whether head or tail of the penny was up. When the first trial of the first 1916 sample was run, the two lines from a single pedigree were carried back simultaneously, as it was often possible to look up two pedigrees in a single volume of the flock book.

Evidently the person doing this work often laid a penny on the table, determined a sequence from it, and then in looking up the next sequence in the other line forgot that the penny lying there had already been used in determining the previous sequence. Thus he often used a single penny throw for two sequences from the same volume of the flock book and in the two lines running back from a single pedigree. Part of the evidence for this is the fact that in this trial an unusual number of ties were found on very recent ancestors in pedigrees taken from consecutive or almost consecutive pages of the flock book, and on ancestors which did not often appear at all in other pedigrees. In the Rambouillet flock book there are often several consecutive pages of pedigrees registered from a single flock. If in such a flock the breeder was rather extensively using the son of one ram on daughters or granddaughters of the same ram, and if the person tracing the two lines simultaneously from the same pedigree often forgot and used the same penny throw a second time, the results actually found would have been almost inevitable. Additional evidence in favor of this explanation comes from the fact that others working with this method in this laboratory often found themselves consciously in doubt as to whether they had just used or were just getting ready to use a given penny in the tracing of these lines. When the worker was aware of this, the doubtful penny was discarded, but a worker not constantly on guard might often use the same penny throw twice. The systematic error that would result would not affect the relationship coefficients materially. Of course there is no reason to expect often such remarkably close agreement as was actually observed between the other three 1916 trials.

RELATIONSHIP COEFFICIENTS

On the advice of Doctor Wright a slight departure was made from the method outlined by Wright and McPhee.[3] In calculating the inter se relationship within the sample of 400 pedigrees, one line from one pedigree was matched with another line chosen at random from among all the other pedigrees. When a line was once used in this matching process, it was marked and was not again used for this purpose. Thus from four hundred 2-column pedigrees there were 400 matchings of one line against one line, no line being used a second time.

[3] Wright has called attention to an error in the printed statement of Wright and McPhee's method in the twelfth line on p. 383 which reads: "A tie in a comparison of four-column pedigrees (four possible ties) * * *." The word "four-column" should be "two-column."

The relationship of individual animals to the entire breed is made up partly of direct relationship and partly of collateral relationship. For example, a given animal, B, may be found as an ancestor in 16 out of a possible 800 lines in a sample. That is direct relationship. In addition to this, B's sire and dam may perhaps be found in 12 more lines which do not trace to them through B but through some of B's half or full brothers or sisters. This would bring about a certain amount of genetic resemblance between B and the entire breed by collateral relationship. More remote ancestors may also contribute to this relationship, although of course the additional contribution made by another collateral appearance of an ancestor is approximately halved with each generation farther back the ancestor is in the pedigree.

The probable errors for the coefficient of relationship between an individual and the entire breed will depend in part on how much of the relationship is direct and how much is collateral. The collateral relationship is spread out over a larger number of ancestors, and the probable error contributed by finding or failing to find a particular ancestor decreases as that ancestor becomes more remote. The maximum probable errors for these relationship coefficients occur where all the relationship is direct. The following probable errors are approximately correct if one-half of the total relationship found is direct, one-fourth of the total amount is collateral through the two parents, and the remaining one-fourth is collateral through the four grandparents. On the whole, this gives probable errors that are smaller than they should be for most of the individual animals shown in Table 1. Consequently the differences between the coefficients from duplicate samples in Table 1 will, when compared with these probable errors, appear more significant than they really are. The approximate probable error of the difference between two coefficients of relationship, each calculated from a sample of 800 random lines, is as follows:

Size of coefficient	Probable error of difference between two such coefficients	Size of coefficient	Probable error of difference between two such coefficients
Per cent		*Per cent*	
2	0.40	6	0.68
3	.48	7	.73
4	.55	8	.78
5	.62	9	.82

When these approximate probable errors are compared with the 60 differences between the coefficients for the same animal in duplicate samples, 22, or 37 per cent, of those differences are less than these probable errors; 35 per cent of them exceed twice these probable errors, and 5, or 8 pr cent, of them exceed three times these probable errors.

The probable errors were calculated exactly for these five largest differences, and it was found that two were really less than three times their probable errors, while the other three (20079 in 1916, 1261 in 1916, and 13405 in 1926) were 3.4, 3.7, and 4.0 times their respective probable errors. In a group of 60 differences one would expect two or three to exceed the limits of three times their probable errors just as a matter of chance.

Figure 1 shows graphically the agreement between the number of times ancestors were found in the first and second 1926 samples. All ancestors found as many as five times in either sample are included. Those not found five times in at least one sample are certainly not important. In general, close agreement is evident. Since the number of times an ancestor is found is not entirely independent of the number of times each of its descendants or ancestors is found, it was not thought worth while to reduce the facts shown in Figure 1 to mathematical terms.

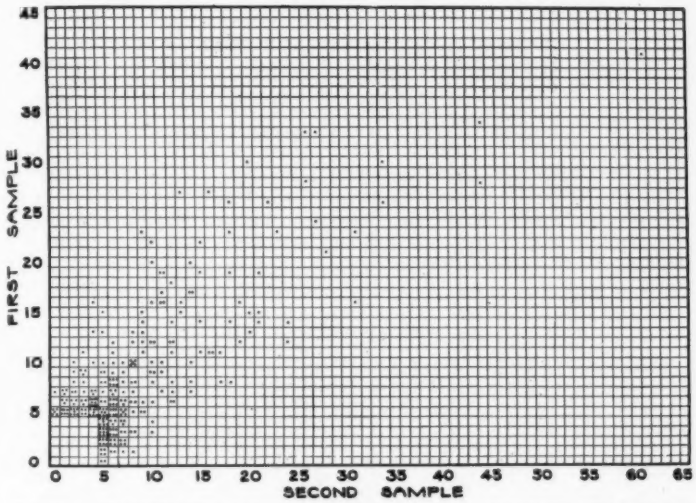


FIGURE 1.—The relation between the number of times an ancestor was found among the 800 random lines in the first 1926 sample and the number of times that the same ancestor was found in the 800 random lines of the second 1926 sample. Ancestors not found as often as five times in either sample have been admitted

CONCLUSION

These empirical tests of the method of Wright and McPhee lead to the conclusion that the method is about as accurate as its theoretical probable errors indicate, if all sources of systematic error which might prevent the lines from being truly random are carefully avoided. One must be continually on guard, however, against systematic errors which at first thought appear to be so trifling as not to be worth attention, but which under certain circumstances may creep into the supposedly random tracing of the lines.

OXIDATION-REDUCTION POTENTIALS AND THE HYDROGEN-ION CONCENTRATION OF A SOIL [1]

By L. G. Willis

Soil Chemist, North Carolina Agricultural Experiment Station

INTRODUCTION

The oxidation-reduction equilibrium of soils has had scant attention from those interested in soil problems. There can be no doubt that it constitutes an important soil property, yet the publications of Gillespie [2] and of Remezow [3] report the only formal investigations of the problem.

A number of observations made in the course of experimental work in eastern North Carolina pointed rather definitely to the conclusion that liming increased the reductiveness of those soils characterized as being naturally poorly drained, strongly acid, and high in organic matter. Some preliminary work showed that reproducible oxidation-reduction potentials could be obtained with one of these soils provided certain details of manipulation were followed. On the basis of this work a method was devised and the study of the effect of reaction on soil potential was undertaken.

EXPERIMENTAL DATA

EFFECT OF LIMING A SOIL ON THE OXIDATION-REDUCTION POTENTIAL

The soil selected for the work was a Dunbar fine sandy loam, one of a group of naturally poorly drained soils of the lower coastal plain in North Carolina. Five lots of this soil were limed at different rates and stored in 1-gallon glazed pots at 10 per cent moisture content for several months. They were then air-dried, the lumps were crushed, and samples for use were kept in glass-topped jars during the work.

Air-dry storage was adopted because a better-established equilibrium was maintained thereby, and also because it permitted greater facility in manipulation. Another possible advantage lay in the exposure of the soil to air while drying in order to oxidize as far as possible any reducing compounds formed by microbial action during incubation.

In preparation for the determination of the oxidation-reduction potential, 50 grams of the air-dry soil was shaken for three days in an unstoppered 250 c c volumetric flask of pyrex glass with 50 c c of distilled water in a constant-temperature air bath at 35° C. Erratic results obtained by shaking the sample at room temperature made it seem advisable to maintain a constant temperature during this operation.

[1] Received for publication Mar. 8, 1932; issued November, 1932. Contribution from the Agronomy Department, North Carolina Agricultural Experiment Station, as Paper No. 57 of the Journal Series.

[2] Gillespie, L. J. Reduction potentials of bacterial cultures and of water-logged soils. Soil Sci. 9: 199–216, illus. 1920.

[3] Remezow, N. P. Die oxydierenden und reduzierenden Prozesse in dem Podsolboden. Ztschr. Pflanzenernähr., Düngung u. Bodenk. 15:34–44. 1929.

On the basis of the results shown in Table 1, the 3-day period of shaking the samples was adopted. The potentials recorded are from duplicate determinations and are calculated with reference to the normal hydrogen electrode.

TABLE 1.—*Oxidation-reduction potentials of soil-water suspension in duplicate determinations*

Period of shaking	Potentials	
	Volt	*Volt*
½ hour	+0.352	+0.383
1 day	.413	.409
2 days	.424	.435
3 days	.436	.436
4 days	.431	.436

The entire contents of the flask in which the samples were shaken were transferred to a specially constructed electrode flask illustrated in Figure 1.

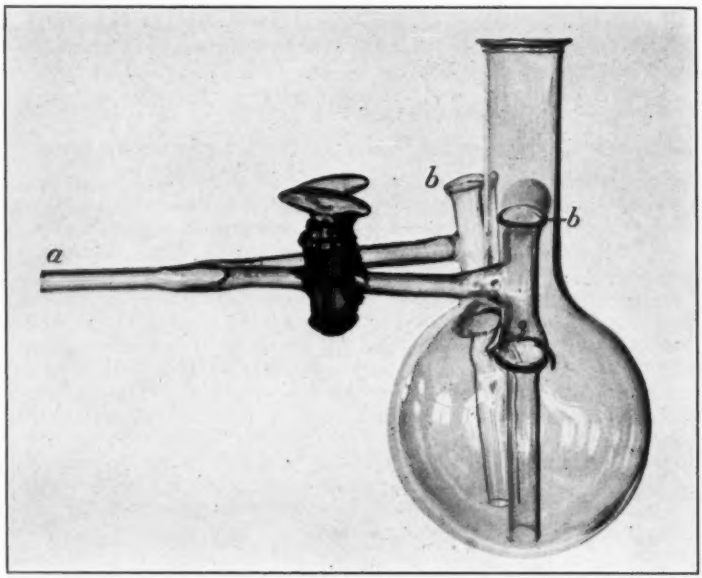


FIGURE 1.—Electrode flask for soils: *a*, Connection for nitrogen supply; *b*, electrode tubes

This was made by fusing two glass tubes into the side wall of a 300 c c round-bottom pyrex-glass flask in such a way that they reached nearly to the bottom inside the flask. Platinum foil electrodes about 5 mm square attached to glass tubing were inserted into these tubes and were held in place about 15 mm from the bottom by means of tightly fitting rubber stoppers at the tops of the tubes. The two tubes provided for duplicate readings from independent electrodes.

The electrode flask containing the soil-water mixture and with the electrodes in place was put into a controlled-temperature water bath at 35° C., and the air was withdrawn through the neck of the flask by means of an efficient vacuum pump, while the stopcocks leading into the electrode tubes were closed. After five minutes the stopcocks were opened enough to permit slow aspiration at reduced pressure with pure nitrogen gas. This was obtained by passing commercial tank nitrogen through an oxygen-absorption apparatus similar to the one described by Kendall.[4]

Aspiration was continued for 10 minutes, then the connection to the vacuum pump was closed and the electrode flask filled with nitrogen. The cocks admitting the nitrogen were then closed, and a partial vacuum was drawn, which was released with air at the neck of the electrode flask. This forced the soil-water suspension into the electrode tubes, covering the electrodes completely. The position of the electrodes in the tubes protected them from contact with oxygen either directly or by diffusion after the flask was opened.

After the electrodes had been immersed in the soil-water suspension 10 minutes a salt bridge from a saturated calomel cell was inserted through the neck of the flask to the soil suspension in the bottom and the potential difference read. Five minutes later another reading was taken.

There was usually a fair agreement between these readings, but since the change was usually toward a better conformity between the two electrodes at the second reading only the latter is reported. Successive readings over a period of several hours showed a slow drift toward a lower potential, but the differences between soils having different treatments were always approximately of the same order.

No attempt was made to test the necessity for removing the oxygen, which was done as an extra precaution to eliminate as far as possible all causes of erratic results. The potentials could hardly have been influenced by oxygen, as a calculation shows that the oxygen pressure as indicated by the potentials and pH values was approximately 10^{-30} atmospheres.

TABLE 2.—*The effect of liming on the oxidation-reduction potential of a soil*

Rate of liming in pounds per acre	Potential			Reaction
	Electrode A	Electrode B	Average	
	Volt	*Volt*	*Volt*	*pH*
None	+0.433	+0.433	+0.433	5.14
1,000	.415	.419	.417	6.10
2,000	.352	.344	.348	6.53
4,000	.302	.304	.303	7.65
6,000	.296	.299	.298	7.72
8,000	.296	.298	.297	7.75

The potentials given in Table 2 were read from duplicate electrodes in the same soil-water suspension. They are in virtual agreement with several other series run on the same samples in preliminary trials of the method.

Reactions were determined by means of the hydrogen electrode.

The evidence appears conclusive that liming decreased the oxidation-reduction potential of this soil. The effect is undoubtedly a

[4] KENDALL, E. C. THE REMOVAL OF TRACES OF OXYGEN FROM NITROGEN. Science (n. s.) 73: 394-397, illus. 1931.

complex one, but there is some support in these results for the opinion that the lower potentials with the higher rates of liming are largely due to decreases in the hydrogen-ion concentration.

The evidence is not conclusive, however, because of the many possible effects of liming other than the modification of hydrogen-ion

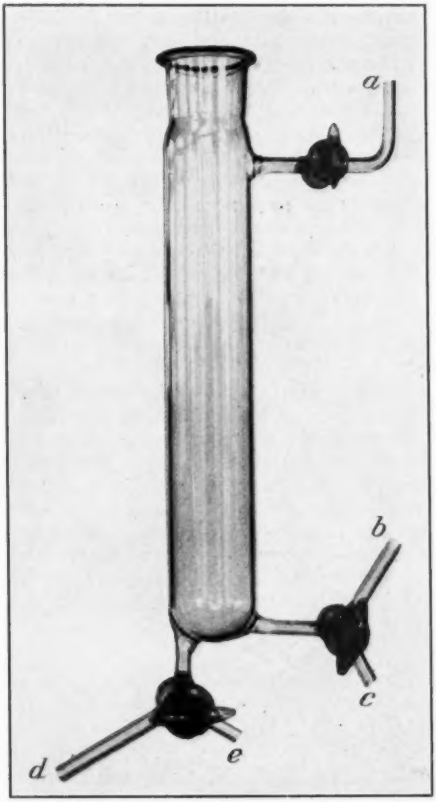


FIGURE 2.—Electrode tube for soil solution: *a*, Outlet to vacuum line; *b*, connection for nitrogen supply; *c*, outlet for withdrawing samples for pH determination; *d*, calomel-electrode contact; *e*, outlet for flushing salt-bridge contact

concentration. A closer approach to proof is found in some potentials obtained in a soil solution, the reaction of which was changed by titration with hydrochloric acid.

RELATION BETWEEN THE REACTION AND OXIDATION-REDUCTION POTENTIAL OF A SOIL SOLUTION

A sample of the soil used in the other experiments was mixed with calcium carbonate at the rate of 5 tons per acre and stored for several weeks at a moisture content of 15 per cent. This was then packed into an 8-liter glass percolater and the soil solution displaced with

water. About 150 c c of this solution was caught in the apparatus illustrated in Figure 2 from which the air had been displaced by nitrogen.

Oxygen was eliminated as before by reduced pressure and replacement with nitrogen, and potentials were read from duplicate electrodes placed in the apparatus near the bottom. Small portions of the solution were withdrawn for the determination of hydrogen-ion concentration, and then one-hundredth normal hydrochloric acid was added from a burette. The solution was well stirred by aspiration with nitrogen and the determinations repeated.

The results (fig. 3) show an inverse linear relation between oxidation-reduction potential and pH value.

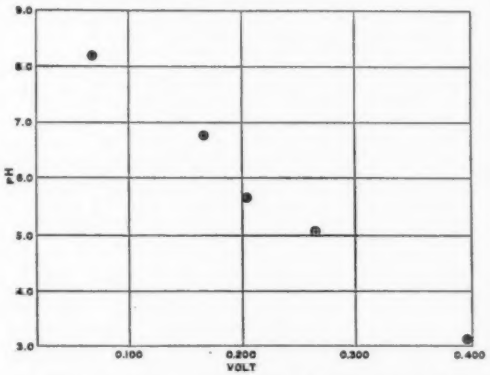


FIGURE 3.—Oxidation-reduction potentials of a soil solution at different pH values

DISCUSSION

The results reported in this paper are not in agreement with those of Remezow, who showed that limed soils had higher potentials than unlimed soils. Since the limed soils in the work he reported received applications of manure also and since the lime was applied several years prior to the determination of the potentials, his results are hardly comparable with those reported here.

Many applications of the data to recognized soil phenomena are suggested, but a discussion of these would necessarily be based on assumptions that are capable of proof.

SUMMARY AND CONCLUSIONS

The oxidation-reduction potential of a soil characterized as naturally poorly drained, strongly acid, and high in organic matter varies inversely with the pH values produced by liming.

A similar result is observed when the displaced solution from the limed soil is titrated with very dilute acid.

Within the category of soils where this relation between oxidation-reduction potential and hydrogen-ion concentration holds, rates of liming must be gauged with reference to the possible effects of the increase in reductiveness of the soil at the higher pH values.

U. S. GOVERNMENT PRINTING OFFICE: 1932